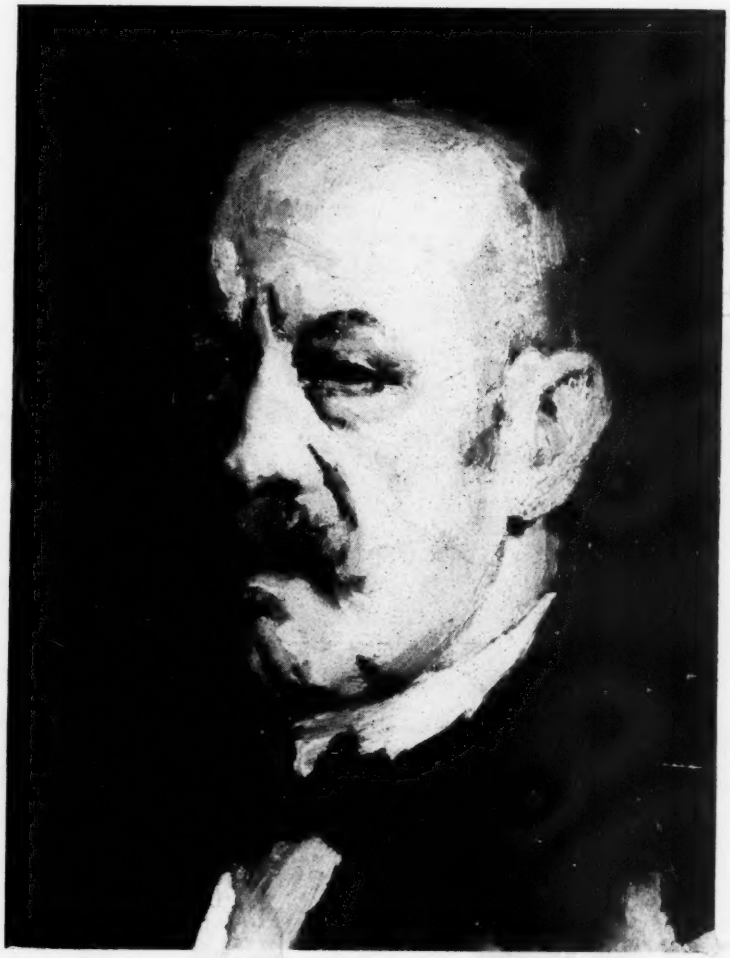
DIABETES

The Journal of the American Diabetes Association



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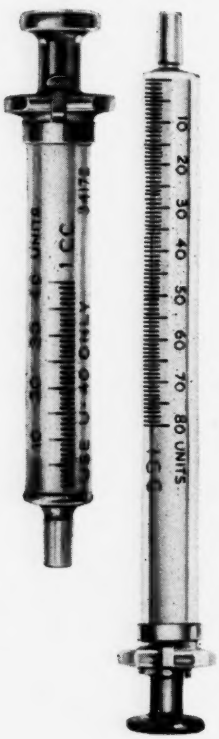

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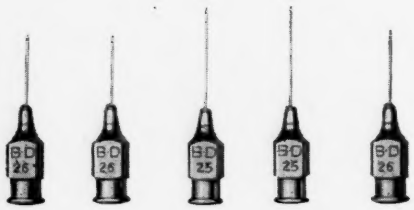

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Studies on the Disposition of Blood Glucose

A Comparison of Insulin and Orinase

James Ashmore, Ph.D., George F. Cahill, Jr., M.D., A. Scott Earle, M.D., and S. Zottu, B.A., Boston

INTRODUCTION

The aromatic sulfonylureas elicit a hypoglycemic response similar to insulin when administered to normal animals. In order to learn more of the mechanism by which the blood glucose is lowered, the effects of Orinase and insulin on the disposition of blood glucose in normal dogs and rats have been compared.

PORTAL-HEPATIC SUGARS IN UNANESTHETIZED DOGS

Preparation of animals. Mongrel dogs weighing from 17 to 21 kg. were anesthetized with intravenous Nembutal, 30 mg./kilo. Respiration was maintained during the intrathoracic portion of the procedure using a positive pressure pump and endotracheal tube. Aseptic technic was maintained throughout the surgical procedure.

The operation has been described in some detail since it has proved a useful and successful technic in studying hepatic physiology. The upper abdomen was entered through a short midline incision, the spleen removed, and the splenic artery and vein isolated. Two cannulae (consisting of a tip of 205 gauge polyethylene tubing 12 cm. long joined to a 60 cm. length of nonkinking "Genflex" plastic tubing by a larger gauge polyethylene cuff) were threaded into the splenic artery and vein and guided respectively into the aorta and portal vein. The cannulae were sutured in place to the cut ends of the splenic vessels through a heat-flared polyethylene cuff. In this way the less reactive polyethylene tip lay within the vessel. The cannulae were filled with dilute heparin, closed at the distal ends with variously colored aluminum plugs, and passed subcutaneously over the right rib margin and beneath the skin of the right lateral thorax, where they were found during the subsequent thoracotomy. The abdominal wound was closed and the animal placed in the left lateral position.

During the second step the right chest was entered, excising the lowermost rib joining the sternum. After retracting the lung, the inferior vena cava was carefully dissected free where it pierces the diaphragm to enter the chest. The largest hepatic vein was identified at the entrance to the cava from the left. The cava, opposite the junction with this vein, was caught in a miniature caval clamp and incised. Fine cotton mattress sutures were placed on both sides of the venotomy. A cannula similar to those previously described but with a 6 cm. tip, and with a malleable copper-wire obturator inside, was threaded into the cava through the venotomy incision while the mattress sutures were held taut to prevent blood loss. The cannula was guided into the largest left hepatic vein and the mattress sutures tied. The tip of the cannula lay approximately 4 cm. within the liver so that the possibility of contamination with caval blood was minimized. The obturator was removed and the cannula filled with dilute heparin and plugged. The thoracotomy wound was closed and the three cannulae were threaded subcutaneously to the midback between the scapulae, through the skin, and into a plastic box held by wire sutures (figure 1).

Six hours later, and then twice daily, the cannulae were filled with heparin which gradually diffused into

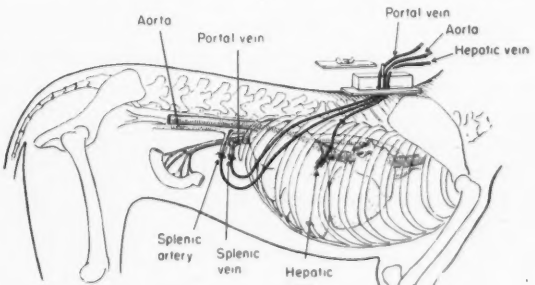

FIG. 1. Schematic drawing showing the technic used in arterial, portal and hepatic vein cannulae in dogs.

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the circulation to provide local and systemic heparinization. The animals were given penicillin and streptomycin postoperatively. Blood samples could be collected from such cannulae at any time, intra- or postoperatively, to study whatever facet of liver physiology desired.

Portal-hepatic changes. Experiments were begun on the third to fourth day following operation and done in the following order: On the first experimental day a glucose load was given portally and the hepatic, portal and arterial samples drawn simultaneously at 5, 15, 30, 45 and 60 minutes. The cells were immediately separated and the plasma analyzed for glucose, potassium and inorganic phosphate. Glucose was determined by the method of Nelson[1] on Somogyi[2] filtrates of the plasma; potassium was measured on an internal standard flame photometer, and inorganic phosphate by the method of Fiske and Subbarow.[3] The glucose tolerance of these dogs was found to be normal in all cases.

On the second day, glucagon was injected via the portal catheter and the study repeated. These tests were performed to ascertain that the catheters were in place and operating satisfactorily. On the third day, 7 units of glucagon-free insulin (Lilly) were given intraportally and the experiment repeated. On the fourth day, 500 mg. of sodium Orinase (Upjohn) was injected and the experiment again repeated.

The effects of insulin and Orinase are compared in figure 2. Dog 3 represents a typical experiment in a series of four animals studied. Both substances caused a rapid fall in portal and hepatic glucose, and comparable degrees of hypoglycemia were obtained in all experiments. It was observed that in the case of hypoglycemia produced by insulin, hepatic glucose levels were always appreciably higher than portal values, but following Orinase there was a corresponding fall in both portal and hepatic glucose.

Portal-hepatic differences have been plotted in figure 3 as a function of time following insulin (top curves) and following Orinase (bottom curves). After insulin, the liver released glucose into the hepatic venous blood, and potassium and inorganic phosphate were initially taken up and then released as hypoglycemia persisted. No significant portal-hepatic differences in glucose, potassium or inorganic phosphate were observed with Orinase.

In figure 4, portal glucose concentration has been plotted as a function of hepatic glucose concentration. These points represent all analyses from a series of four dogs. The line at 45° indicates equal glucose in portal and hepatic plasma. All points above this line would represent concentrations of hepatic glucose greater than

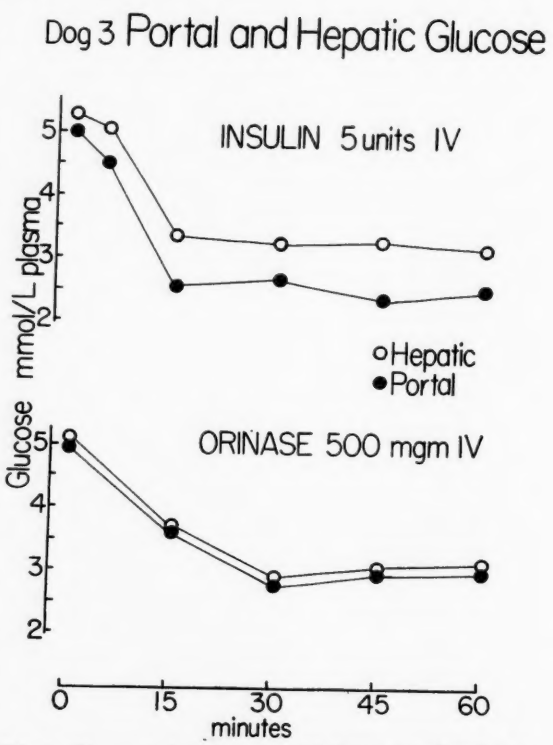

FIG. 2. Changes in hepatic and portal glucose are plotted as a function of time following injection of 5 units of glucagon-free insulin (top curves) and 500 gm. sodium Orinase (lower curves). Dog 3 represents a typical experiment in a series of four animals studied.

portal glucose; all points below the line represent higher portal than hepatic glucose. Insulin hypoglycemia (open circles) appears to result in hepatic glucose release; conversely, there was no evidence of hepatic glucose production after Orinase (closed circles) and it appears that the liver removes glucose from the circulation. Similar results on the effect of Orinase on portal and hepatic glucose have previously been reported by Purnell et al.[4]

Calculation of hepatic glucose production. Assuming that the hepatic blood flow does not change appreciably after insulin and Orinase administration, hepatic glucose production has been calculated on the basis of a maximal and a minimal hepatic blood flow of 3 and 1 liters/kilo./hour.[5] Such calculations have been summarized in tables 1 and 2. In a typical unanesthetized dog (table 1) blood samples drawn over the period of one hour indicate an average glucose production of 6.4 mg./kilo./min., varying between 4.4 and 8.7 mg./kilo./min. for mean values. Individual samples drawn from a series of five animals (table 2) show an average produc-

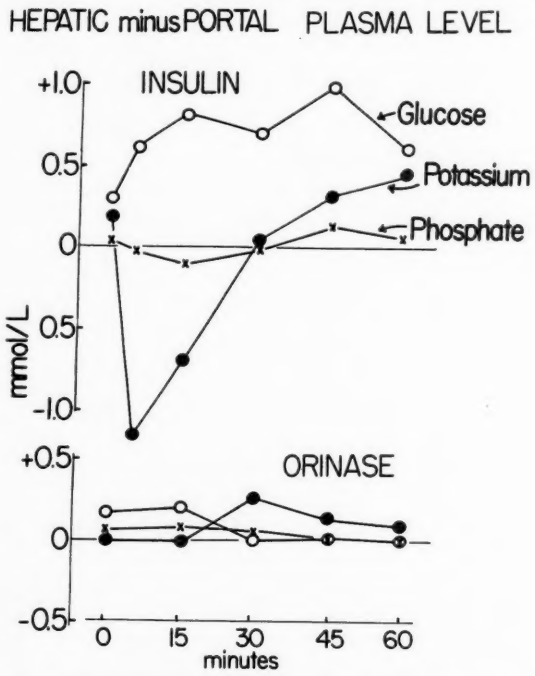

FIG. 3. Hepatic-portal plasma differences in glucose (o), potassium (●) and phosphate (x) are plotted following injection of 5 units of insulin (top curves) and 500 mg. sodium Orinase (lower curves). These data were obtained on Dog 3 and represent a typical experiment in a series of four animals studied.

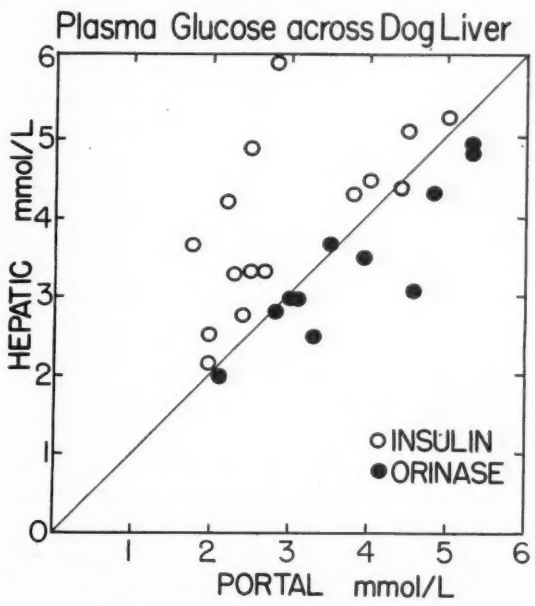

FIG. 4. Hepatic plasma glucose in mmoles/L. has been plotted against portal plasma glucose. These points were obtained by withdrawal of simultaneous portal and hepatic blood samples in a series of four dogs with portal and hepatic vein catheters. Samples obtained after insulin injection (o): after Orinase (●).

tion of 6.1 mg./kilo./min. and vary between 2.4 and 9.6 mg./kilo./min. After insulin injection, hepatic glucose production continues at a normal or slightly accelerated rate; while after Orinase, hepatic glucose production appears to be inhibited. Values for glucose production after glucagon (0.05 mg./kilo. Lilly) are presented for comparison. In this case there was a threefold increase in hepatic glucose production.

It should be pointed out that Orinase injection may be associated with an alteration in blood flow, as suggested by Purnell et al.[4] Even if the flow were to change, this would not alter the interpretation of our data since there was no evidence of any hepatic glucose production in the Orinase injected animals.

Hepatic glucose production in normal and depancreatized dogs has previously been determined by Lipscomb and Crandall[5,6] using London cannulae[7] for portal and hepatic sampling. Values for normal hepatic glucose production obtained by these investigators were lower (2 mg./kilo./min.) than those obtained in this study. One possible explanation for this difference might be that their animals were starved for several days while the dogs used in this study were without food for only twelve to fifteen hours prior to the experimental period.

DISTRIBUTION OF BLOOD GLUCOSE IN RATS

Preparation of animals. Normal male rats of the Wistar strain weighing 200 to 250 gm. were used in these studies. The animals were lightly anesthetized with ether, the femoral vein exposed, and 1 mg. (1.8μ Curies) of randomly labeled glucose-C^{14} injected in 0.5 ml. saline. When the effects of insulin and Orinase were studied, these compounds were injected in the same solution with the glucose. Only glucagon-free insulin (Lilly) was used and at a dose of 0.25 units/animal. Sodium Orinase (Upjohn) was given at a dose of 20 mg./animal.

Blood samples (0.2 ml.) were obtained from the tail at 5, 15, 30, 45 and 60 minutes after injection. A single Somogyi filtrate was prepared and 1 ml. of this filtrate analyzed for reducing sugar. Carrier glucose (10 mg.) was added to another aliquot of the filtrate and glucose isolated as the phenylosazone. This was recrystallized, plated and assayed for C^{14}.

Specific activity of blood glucose. When the logarithm of blood glucose specific activity was plotted as a func-

TABLE 1

Hepatic glucose production in a normal unanesthetized dog over a period of one hour

Time	Blood glucose Portal	Hepatic Arterial		Hepatic Glucose Output Max.	Min.	Mean
	(mg./100 ml.)			(mg./kg./min.)		
	No injection					
9:00	104	115	104	6.6	2.2	4.4
9:20	104	126	114	13.0	4.4	8.7
9:40	115	128	120	7.8	2.6	5.2
10:00	109	127	106	11.0	3.6	7.3
					Average	6.4
	After insulin intraportally					
9:00	90	96	92	3.6	1.2	2.4
9:05	81	91	87	6.0	2.0	4.0
9:15	45	60	47	9.0	3.0	6.0
9:30	47	59	48	7.2	2.4	4.8
9:45	42	60	48	11.0	3.6	7.3
10:00	44	56	47	7.2	2.4	4.8

TABLE 2

Hepatic glucose production in normal unanesthetized dogs after insulin, Orinase or glucagon

Blood glucose Portal	Hepatic Arterial		Hepatic Glucose Output Max.	Min.	Mean
(mg./100 ml.)			(mg./kg./min.)		
Uninjected					
108	119	116	6.6	2.2	4.4
90	96	92	3.6	1.2	2.4
99	119	102	12.0	4.0	8.0
80	104	83	14.0	4.8	9.6
104	115	104	6.6	2.2	4.4
				Average	6.1
Sixty minutes after insulin					
39	76	47	22.0	7.4	15.0
68	78	76	6.0	2.0	4.0
42	60	48	11.0	3.6	7.2
				Average	8.6
Sixty minutes after Orinase					
70	63	68			0
38	35	34			0
55	55	54			0
				Average	0
Fifteen minutes after glucagon					
216	252	218	22.0	7.2	14.0
130	155	131	15.0	5.0	10.0
200	282	270	49.0	16.0	33.0
				Average	19.0

tion of time in fed normal rats after injection with saline and glucose-C^{14}, a typical linear decay curve was obtained (figure 5). When insulin and glucose-C^{14} were injected, the decay curve was more rapid. The fall in specific activity resulted from a dilution with unlabeled glucose. When sodium Orinase was injected, the fall in specific activity was identical with that of the saline-injected controls. The same degree of hypoglycemia was produced by insulin and Orinase.

Cori and Cori[8] have demonstrated that insulin hypoglycemia causes epinephrine release from the adrenal medulla and glycogen breakdown. We had reasoned that if this were the case, dilution of blood glucose could be minimized by using fasted rats. The experiments were therefore repeated in animals that had been fasted for twenty-four hours prior to the injection. The results of such experiments are given in figure 6. Once again, insulin caused a greater dilution than that obtained with Orinase or saline. As in the previous experiments, the same degree of hypoglycemia was produced by both insulin and Orinase.

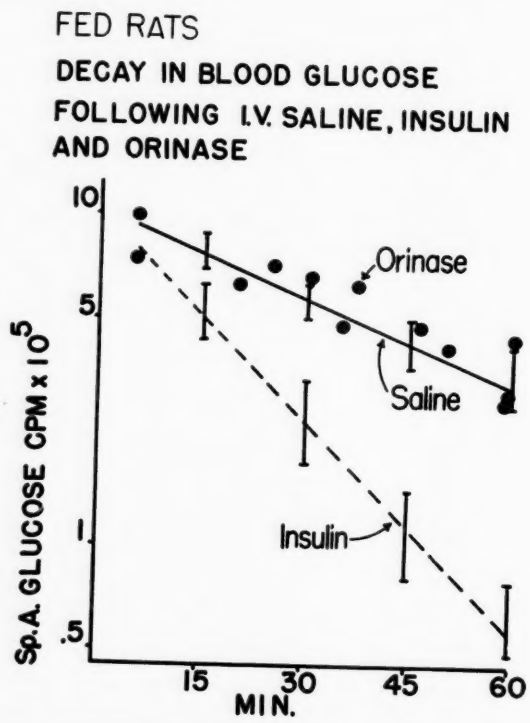

FIG. 5. Log. specific activity of blood glucose has been plotted as a function of time in variously treated fed normal rats after injection of 1 mg. 1.8 μC glucose-C^{14}. Each curve represents the mean of three experiments. Total spread of points is indicated (I). Individual points following Orinase injection (●) have been plotted.

The experiments were next repeated in animals that had been adrenalectomized four hours previous to the injection of labeled glucose. Such a time arrangement was selected in order to minimize any effect of diminished adrenocortical function. The results of these experiments are given in figure 7. Although insulin given to adrenalectomized animals still resulted in a more rapid fall in blood glucose specific activity than that observed in the saline-injected controls, the dilution effect

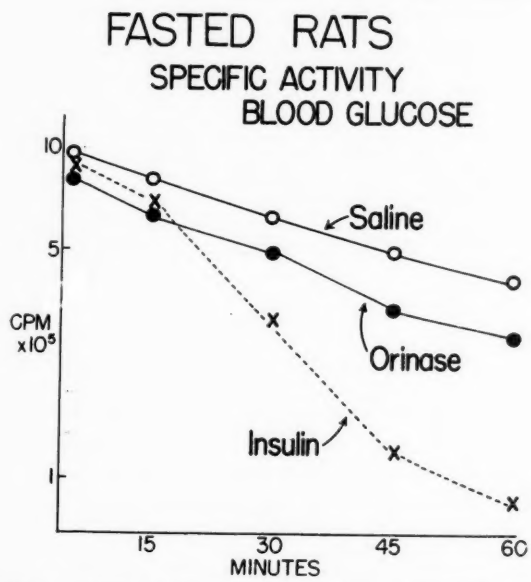

FIG. 6. Changes in specific activity of blood glucose after injection of saline (o), 20 mg. of Orinase (●) and 0.25 units of insulin (x) to fasted rats are shown. Conditions are identical with those given in figure 4.

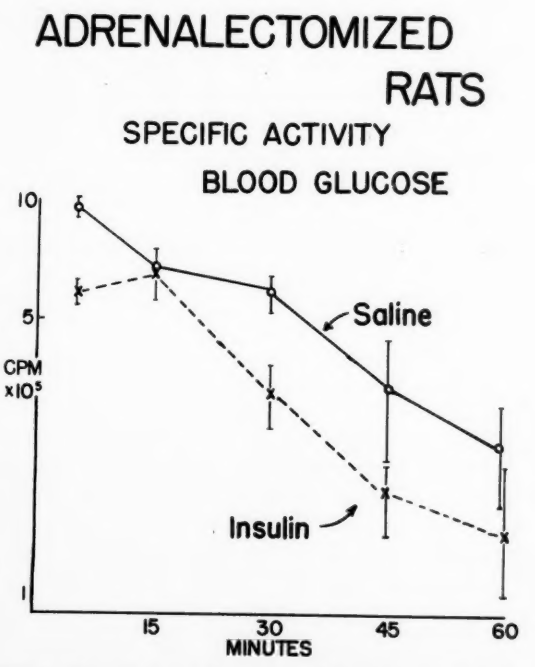

FIG. 7. Changes in specific activity of blood glucose after intravenous administration of saline (top curve) and insulin to adrenalectomized rats. Each curve represents the mean of three animals and total spread of points is indicated.

observed was not as marked as that in normal animals.

Effect of insulin and Orinase on liver and muscle glycogen. Male rats, 220 to 250 gm., were fasted for twenty-four hours and injected as described in the preceding section with glucose-C^{14}, plus either insulin, Orinase or saline. At the end of one hour the animals were stunned, exsanguinated, and liver and muscle samples removed and placed in hot 30 per cent potassium hydroxide. Tissue glycogen was isolated and hydrolysed to glucose[9] which was determined colorimetrically[1] and assayed for C^{14} as the phenylglucosazone.[10] The results of these experiments are given in table 3. The animals injected with insulin had deposited very little of the C^{14} in liver glycogen. Orinase-injected animals, however, deposited as much, if not significantly more C^{14} in liver glycogen than the saline-injected controls. In the case of muscle, insulin resulted in appreciably more C^{14} deposition into glycogen than did Orinase. Again both insulin and Orinase produced the same degree of hypoglycemia.

TABLE 3

Action of insulin and Orinase on the incorporation of C^{14} glucose into liver and muscle glycogen in fasted normal rats

Injection	Liver Glycogen		Muscle Glycogen	
	μM./gm.	cpm./gm.	μM./gm.	cpm./gm.
Saline	13	1,450	34	19
	3	585	18	19
Insulin	3	9	44	320
	9	25	11	242
Orinase	15	2,100	12	52
	6	2,200	16	43

Although the hypoglycemia produced in sixty minutes was the same, there was a marked difference in the rate of fall of blood glucose after insulin and Orinase. After intravenous insulin, there was an immediate drop in blood glucose with hypoglycemia continuing for the balance of the sixty-minute period. With Orinase, the glucose concentration fell slowly over the experimental period. In order to obviate effects that might result from this difference in rate of fall of blood glucose, we compared the effects of insulin administered subcutaneously with Orinase injected intravenously. Under these conditions the same rate of blood glucose change was produced by both compounds (figure 8). Changes in the specific activity of blood glucose in these animals are recorded in the lower curves of figure 8. As in previous experiments, the decay of glucose specific activity was quicker with insulin than with saline or Orinase, indicating again a more rapid dilu-

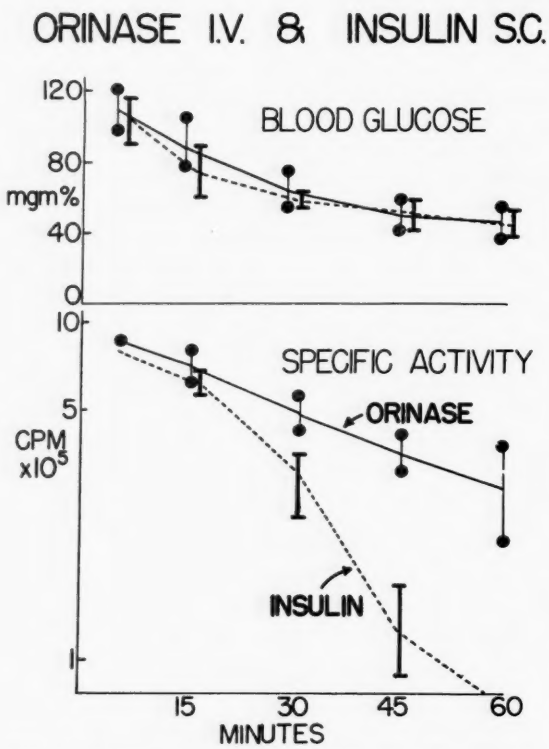

FIG. 8. Changes in concentration of blood glucose (top curves) and specific activity of blood glucose (lower curves) after Orinase (intravenous) and insulin (subcutaneous) administration to fed normal rats. Each curve represents the mean of three animals and total spread of points is indicated.

TABLE 4

Action of insulin and Orinase on the incorporation of C^{14} glucose into liver and muscle glycogen in fed normal rats

Injection	Liver Glycogen		Muscle Glycogen	
	μM./gm.	cpm./gm.*	μM./gm.	cpm./gm.*
Saline (4)	191	100±33	19	29±13
Insulin (5)	88	14±6.3	19	262±59
Orinase (4)	145	143±58	18	61±18

* ±S.E.

TABLE 5

Action of insulin and Orinase on the incorporation of C^{14} glucose into liver and peripheral fatty acids in fed normal rats. All values expressed as cpm./gm. fatty acid

	Liver	Peripheral
Saline (4)	300	375
Insulin (5)	940	1,770
Orinase (4)	1,770	475

TABLE 6

Action of insulin on the incorporation of C^{14} glucose into liver and muscle glycogen of adrenalectomized rats

Injection	Liver Glycogen		Muscle Glycogen	
	μM./gm.	cpm./gm.	μM./gm.	cpm./gm.
Saline	32	24	10	8
	78	42	25	50
	50	17	28	29
Insulin	93	13	20	480
	6	0	21	325
	38	11	33	545

tion of the labeled blood glucose.

Liver and muscle samples were removed at the end of the experimental period and total glycogen and C^{14} content determined. Insulin resulted in little of the C^{14} from the injected glucose being incorporated into liver glycogen, but increased C^{14} incorporation into muscle glycogen (table 4). Although blood glucose fell to the same level with Orinase as with insulin, Orinase did not significantly increase the incorporation of C^{14} from blood glucose into muscle glycogen.

In these animals liver and peripheral fatty acids were recovered after saponification and assayed for C^{14} content. Mean values, expressed as counts/min./gm. fatty acid, are given in table 5. Both insulin and Orinase increased the activity of liver fatty acids, but only insulin increased the activity of peripheral fatty acids.

Values for C^{14} content of liver and muscle glycogen of adrenalectomized rats are given in table 6. Administration of insulin with the labeled glucose did not cause an increase in the activity of liver glycogen, but appreciably increased incorporation of label into muscle glycogen.

DISCUSSION

The experiments with intact dogs have shown that the hypoglycemia resulting from Orinase administration is accompanied by a cessation of hepatic glucose output. The rat experiments corroborate these findings. A fall in specific activity in blood glucose reflects dilution by new unlabeled glucose added to the circulation by the liver. The hypoglycemia after Orinase administration to rats is associated with a fall in blood glucose specific activity which is equal in rate to that of the untreated rat. However, a reduction of the total circulating glucose in the hypoglycemic Orinase treated animal to approximately one-half that of the control would likewise necessitate a reduction in the rate of inflow of un-

labeled glucose from the liver if the activity of circulating glucose were to fall at a normal rate.

On the other hand, insulin administration to dogs resulted in a slightly increased rate of hepatic glucose production. Likewise, the insulin-treated rat shows a markedly accelerated decay of blood glucose specific activity.

A decrease in hepatic glucose production following Orinase administration to normal animals might have one of several meanings. Since the phenomenon of glycogenolysis following insulin hypoglycemia is believed to be mediated by the adrenal medulla,[8] the possibility exists that Orinase in some way prevents a normal hepatic response to epinephrine. Such a conclusion is further strengthened by observations that Orinase will reduce the rate of glycogenolysis in liver slices stimulated by epinephrine.[11, 12] The possibility also exists that Orinase may prevent glucose release from the liver by inhibiting glucose-6-phosphatase or some other enzyme system. In vitro experiments on glucose-6-phosphatase indicate that such a mechanism is most unlikely.[13, 14]

Berson and Yalow[15] have recently reported similar results. Following the disappearance of labeled glucose in rabbits injected with glucose-C^{14}, they concluded that the sulfonylureas produced hypoglycemia without increasing the rate of glucose removal, in contrast to insulin which produced hypoglycemia with an accelerated rate of glucose removal from the blood stream.

Apart from any hepatic mechanism, insulin and Orinase differ in their ability to stimulate peripheral glycogen deposition and lipogenesis. When quantities of insulin and Orinase which produce identical blood glucose changes were compared, insulin markedly stimulated incorporation of C^{14} from labeled glucose into muscle glycogen and peripheral fatty acids. Orinase did not cause a significant increase in C^{14} content of either peripheral glycogen or fatty acids.

SUMMARY

Insulin hypoglycemia in normal dogs is associated with an increased glucose production by the liver. Orinase hypoglycemia is associated with a diminished glucose production and frequently with an uptake of glucose by the liver.

In normal rats, insulin hypoglycemia causes a more rapid disappearance of radioactive glucose as measured by the decay in specific activity of blood glucose. Orinase hypoglycemia is accompanied by a decreased rate of decay in the total activity of circulating glucose.

Recovery of the radioactive carbons from glucose in glycogen and fat, both in the liver and periphery, suggests that insulin provokes incorporation of glucose into muscle glycogen and peripheral fat. Orinase treatment does not produce this effect and must lower blood sugar by other physiologic mechanisms.

SUMMARIO IN INTERLINGUA

Studios in Re le Disposition de Glucosa de Sanguine: Un Comparation de Insulina e Orinase

Hypoglycemia a insulina in canes normal es associate con un augmento del production de glucosa per le hepate. Hypoglycemia a orinase es associate con un diminuite production de glucosa e frequentemente con le acceptation de glucosa per le hepate.

In rattos normal, hypoglycemia a insulina causa un plus rapide disparition de glucosa radioactive, mesurate per le disintegration de activitate specific de glucosa del sanguine. Hypoglycemia a orinase es accompaniate per un relentation del degeneration del activitate total de glucosa circulante.

Le recovration de carbones radioactive ab glucosa in glycogeno e grassia, tanto in le hepate como etiam in le peripheria, suggere que insulina provoca le incorporation de glucosa in le glycogeno muscular e le grassia peripheric. Tractamento a orinase non produce iste effecto. On debe concluder que illo reduce le sucro de sanguine per altere mechanismos physiologic.

ADDENDUM

Since the preparation of this manuscript, "Studies on the Utilization of Uniformly Labeled-C^{14} Glucose by Rats Given Tolbutamide" has been published by W. L. Miller, Jr., J. J. Krake and M. J. Van der Brook (J. Pharm. Expt. Therap. [1957], *119*:513). These authors have compared the effects of insulin and Orinase on the disposition of labeled blood glucose over a six-hour period. They observed that after six hours both insulin and Orinase increased the incorporation of C^{14} from glucose into muscle glycogen. However, in agreement with these studies, only Orinase increased the incorporation of C^{14} into liver glycogen.

ACKNOWLEDGMENT

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Mechanism of Weight Loss by Amphetamine

The use of sympathomimetic drugs as appetite depressants began with the observations of physicians who noted weight reduction as a side effect in patients undergoing amphetamine treatment for other illness.

In attempts to explain this appetite-depressing phenomenon, the following mechanisms have been suggested by various investigators: (1) increased basal metabolism, (2) increased general metabolism, (3) diuresis, (4) incomplete digestion or absorption of food, and (5) reduced food intake from appetite reduction.

In one experiment seven obese and two nonobese subjects were fed three meals a day ad libitum for two eight-week periods, separated by a four-week vacation, and were given either 5 mg. of D- or 10 mg. of DL-amphetamine one hour before meals, during the second half of each eight-week period. Both voluntary caloric intake and body weight were reduced. During the first eight weeks of the experiment, the subjects lost an average of 5.87 lb.—2.44 lb. during the four-week control period, 2.79 lb. during the first week of treatment, and 3.43 lb. over four weeks of medication. In the second eight-week experiment, the results were 2.01 lb. lost during the four control weeks, 2.74 lb. during the first week of medication, and 5.50 lb. over four weeks of treatment.

To assess any toxic or metabolic effects of amphetamine, ten nonobese men were given 3,000 calories per day for fourteen weeks. From day 27 to 56 inclusive, each man received 5 mg. of D- or 10 mg. of DL-amphetamine before each meal. An average weight loss of 0.7 lb. occurred during the control period. On medication, the group averaged, during the first week, a loss of 0.9 lb. but the average loss for the first fifty-six days of treatment was 1.8 lb., only 0.4 lb. less than if the control rate had continued. This is interpreted as indicating an increased total metabolism during the first week of treatment. Supporting this view were reports of wakefulness, during the first week, by eight of the ten subjects.

The mechanism of action of this drug on the central nervous system remains obscure. No significant changes in blood glucose tolerance were observed in human subjects, either immediately or weeks after amphetamine treatment. There was no indication of intoxication. Addition of barbiturates allayed the restlessness of certain patients without inhibiting the anorexia. Thus, the possibility that anorexia was a product of cerebral excitation seems to be eliminated.

The question of whether amphetamine has a specific inhibitory effect upon an "appetite center" or a specific stimulatory effect upon an "appetite inhibiting center" might be answered by testing the drug on carefully prepared subjects.

Tolerance to the anorexigenic drugs often occurs. Some clinicians seem to prefer to use the drugs until they are tolerated, hoping that the patient will have formed new eating habits by that time. Others feel that judicious reduction of the caloric intake of the patient (by as little as 10 per cent) remains the method of choice.

From "Mechanism of Weight Loss by Amphetamine" in *Nutrition Reviews* *14*:71-72, March 1956.

Role of the Adrenal Cortex in Diabetic Retinopathy and Nephropathy

Harold Rifkin, M.D., Samuel Solomon, Ph.D., and Seymour Lieberman, Ph.D.,
New York City

A number of clinical and experimental observations suggest that the adrenal cortical hormones may be involved in the pathogenesis of diabetic retinopathy and nephropathy.[1-3] The evidence that has been marshalled is rather indirect and circumstantial in nature, with little supporting evidence in the field of clinical diabetes. Attempts have been made previously to evaluate the level of adrenal cortical activity in diabetic patients, based on crude measurements of the urinary 17-ketosteroids and 17-hydroxycorticoids.[4-7] These studies have yielded conflicting results. In view of the variable results reported, and since hypophysectomy and total adrenalectomy[8-10] are already being performed for possible alleviation of the capillary vascular complications of diabetes, we have attempted to reassess with newer technics adrenal cortical function in diabetic patients who have been carefully evaluated with regard to the existence, nature and extent of vascular disease.

The problem, confronting us three years ago when this study was first begun, was how to obtain objective evidence of adrenal cortical activity in a clinical state not characterized by the usual criteria associated with increased or decreased adrenal cortical function.[11] It seemed that probably the most direct approach would be to measure the following parameters of adrenal cortical function:

1. The total identifiable and individual urinary 17-ketosteroids.

2. The total urinary 17-hydroxycorticosteroids. Measurements of the individual urinary corticoids, such as Compounds E, F, Tetra-hydro E, Tetra-hydro F, Cortol and Cortolone, are being studied at present and will be the subject of a future report.

3. The plasma 17-hydroxycorticosteroids.

4. The variations produced in these indices by exogenously administered ACTH.

5. In order to evaluate processes involved in the catabolism of steroids, and renal factors governing excretion of the steroidal metabolites, radioactive hydrocortisone was administered intravenously to several patients, and the appearance of radioactivity in the urine was determined at various time intervals.

Presented at the Seventeenth Annual Meeting of the American Diabetes Association in New York City on June 1, 1957.

From the Laboratory and Medical Divisions, Montefiore Hospital, New York City, and the College of Physicians and Surgeons, Columbia University, New York City.

MATERIAL AND METHODS

The present investigation is based on a series of twenty-eight normal patients, ten uncomplicated diabetic patients, fourteen patients with diabetic retinopathy, fourteen patients with the fully developed Kimmelstiel-Wilson syndrome and six nondiabetic patients with various renal diseases. To assure the homogeneity of groups, and to allow comparison of the results obtained, factors such as age and sex were controlled as far as possible by appropriate selection. Renal function was estimated by serial determinations of the blood urea nitrogen, serum creatinine, and endogenous creatinine clearance. Proteinuria was analyzed quantitatively in each twenty-four hour urine sample, and simultaneously analyzed for steroids.

The plasma 17-hydroxycorticosteroids were determined by a modification of the method of Nelson and Samuels.[12, 15] The urinary 17-hydroxycorticosteroids were determined after glucuronidase hydrolysis by the method of Porter and Silber.[13, 14] The individual urinary 17-ketosteroids were analyzed in the following manner:[16, 17, 18] Each urine was hydrolyzed by three methods (glucuronidase, acid *p*H, at room temperature, and acid at 100° C.). The combined neutral extracts from these three hydrolyses were separated by Girard's reagent, yielding a neutral ketonic extract. This ketonic fraction was resolved into its components by gradient elution, chromatography on alumina. The ketosteroids thus isolated were then identified by infrared and

ultraviolet spectroscopy and then estimated by the Zimmermann reaction (figure 1). The C-19-17 ketosteroids can be subdivided into two groups of compounds, those which have an oxygen at C-11 and those which do not, and these will be referred to in the remainder of this report as the C-19-11 oxy and the C-19-11 desoxy compounds, respectively. The C-19-11 desoxy compounds consist of dehydroisoandrosterone, isoandrosterone, androsterone and etiocholanolone, and arise mainly from steroidal precursors which originate either in the adrenals or gonads. The C-19-11 oxy compounds include 11 ketoetiocholanolone, 11 hydroxyandrosterone and 11 hydroxy-etiocholanolone and appear to be derived solely from adrenal precursors (figure 2).

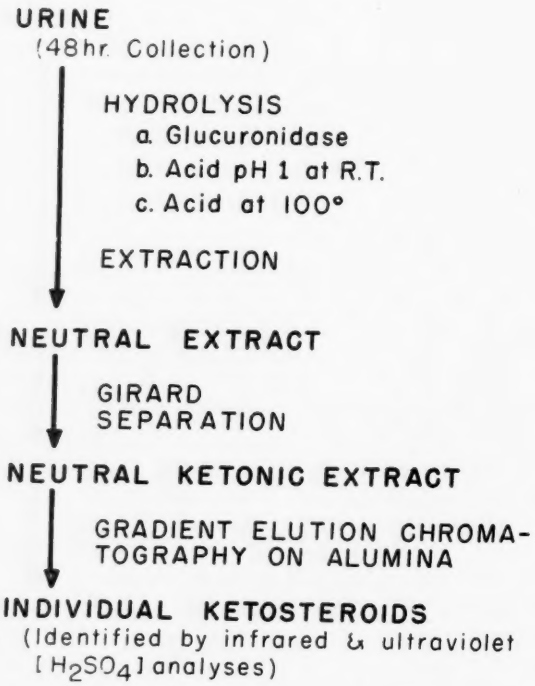

FIG. 1. Method employed for determination of urinary 17-ketosteroids.

RESULTS

The results obtained on the above-mentioned parameters are presented in the accompanying figures.

Figure 3 indicates that the total identifiable ketosteroids, that is, the 11 desoxy- plus the 11 oxysteroids excreted by patients with uncomplicated diabetes are essentially normal, while in patients with diabetic retinopathy and nephropathy, these metabolites vary from relatively normal to low values. In contrast, much

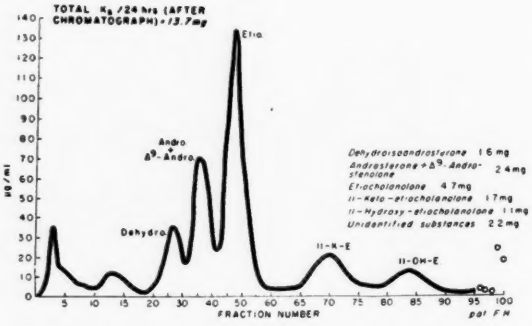

FIG. 2. Chromatogram of patient with diabetic retinopathy.

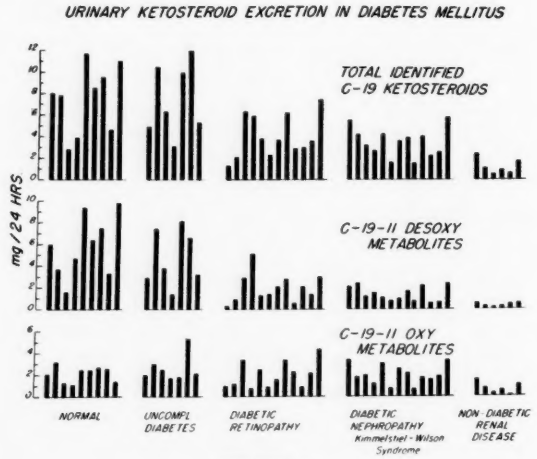

FIGURE 3

smaller quantities of these steroidal metabolites are excreted by nondiabetic patients with renal insufficiency.

Considering the C-19-11 desoxy-metabolites alone, these compounds are excreted by diabetic patients with capillary vascular disease as well as by nondiabetic patients with renal insufficiency in considerably smaller amounts than they are by normal individuals or patients with uncomplicated diabetes.

On the other hand, the C-19-11 oxy-metabolites are excreted by all three diabetic groups in normal or somewhat lesser amounts, whereas this class of metabolites is excreted in diminished amounts by nondiabetic patients with renal insufficiency.

Figure 4 shows the excretion of the urinary 17-hydroxycorticosteroids by a smaller but similar group of patients. The values observed in all diabetic groups are essentially normal.

Figure 5 illustrates the plasma 17-hydroxycorticosteroid levels. No significant deviations from the values

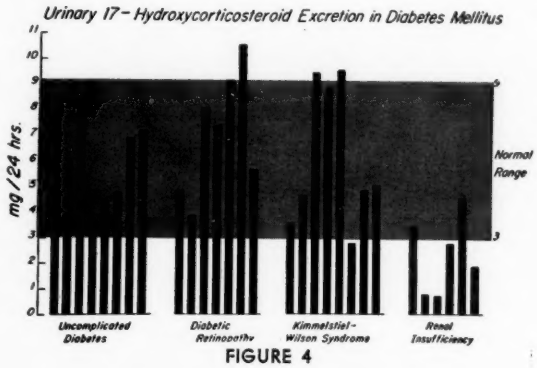

FIGURE 4

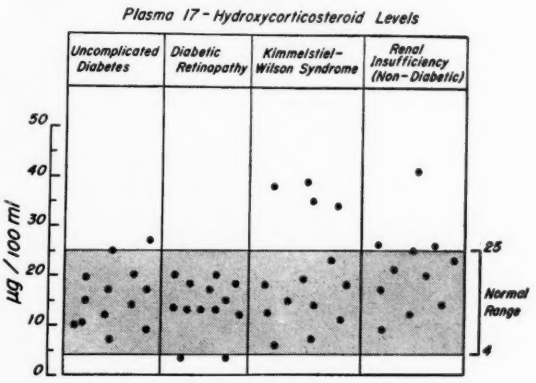

FIGURE 5

obtained in control patients were observed.

The variations produced by the intravenous administration of 40 mg. of ACTH over a six-hour period are shown in figure 6. The plasma 17-hydroxycorticosteroids, the urinary 17-hydroxycorticosteroids, and the urinary ketosteroids, both total and the two sub-groups, namely, the 11 oxy and the 11 desoxy components, rose in a manner quite indistinguishable from that expected in normal control subjects.

Thus, independent parameters of adrenal cortical function do not show evidence of hyperactivity. The fact, however, still remains to be explained that normal amounts of urinary ketosteroids and corticoids are excreted by patients with advanced diabetic nephropathy, whereas markedly diminished quantities are excreted by patients with nondiabetic renal disease.

It is possible that other factors related to alterations of renal function may be responsible for this difference. These factors may include varying degrees of diminished glomerular filtration rate, increased tubular secretion of steroids, and possibly, inhibition of tubular reabsorption of these metabolites.[19, 20] Other factors which have

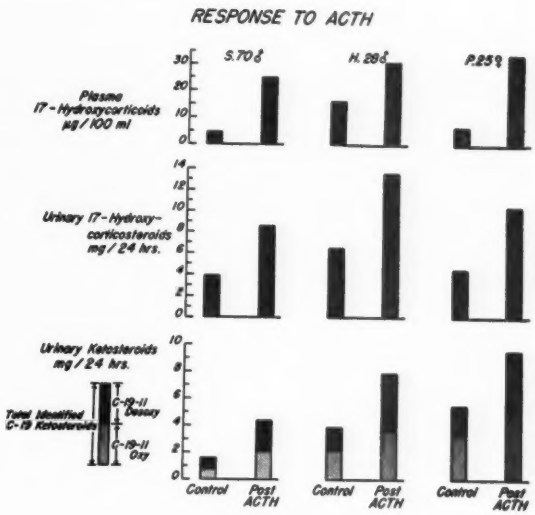

FIGURE 6

recently been considered as influencing urinary steroid excretion are qualitative and quantitative variations in the serum and urinary proteins.[20] In an attempt to evaluate the influence of some of these factors, comparison was made of the excretion of these substances by two groups of patients with diabetic and nondiabetic nephropathy of approximately the same age and sex with similar filtration rates and with similar quantities of proteinuria. Electrophoretic patterns of serum and urinary proteins of both groups were identical. The data indicated that the output of urinary 17-ketosteroids and 17-hydroxycorticosteroids in the patients with diabetic nephropathy still exceeds the amounts found in nondiabetic patients with renal disease.

To test further the role of the kidney, the excretion of radioactive metabolites in the urine following the injection of 4-C^{14} hydrocortisone into five patients was studied. These included three diabetic patients with a fully developed Kimmelstiel-Wilson syndrome, one nondiabetic patient with chronic pyelonephritis and one with chronic glomerulonephritis. These patients were again selected as to be of approximately the same age and sex with similar degrees of azotemia. It can be seen from figure 7 that after twenty-four to seventy-two hours much less of the administered radioactive hydrocortisone appeared in the urines of all these patients than in normal controls.[21, 22] The urinary radioactivity appeared to be closely related to the level of renal function, and no real differences existed between the diabetic and the nondiabetic patient.

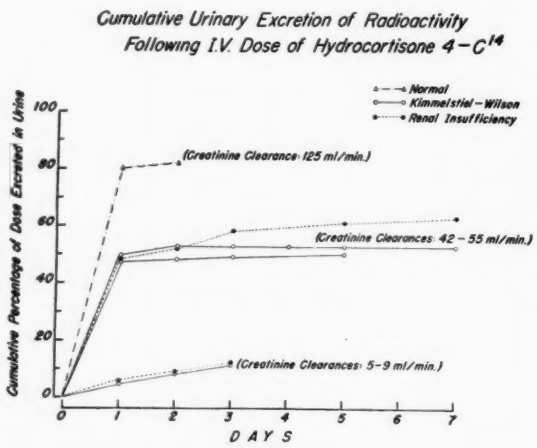

FIGURE 7

DISCUSSION

In spite of these results, which fail to disclose objective evidence of hyperactivity of the adrenal glands of diabetic patients with capillary vascular disease, clinical improvement in the retinopathy and nephropathy following hypophysectomy[9] and adrenalectomy[8] has been reported. This benefit may be totally unrelated to the role of the adrenal and pituitary glands in the pathogenesis of the capillary vascular lesions of diabetes, but may simply reflect nonspecific effects of adrenalectomy and hypophysectomy, which would favorably influence retinal and peripheral edema, hypertension and perhaps proteinuria. It is, of course, conceivable that the methods employed in this study to detect adrenal hyperactivity were inadequate for the task, but this seems rather unlikely. Nevertheless, efforts are now being made by us to estimate the rates of adrenal hormone production by turnover studies utilizing C-14 hydrocortisone, a process which will provide a result independent of the renal and other factors. It is further conceivable that even the normal secretion of adrenal hormonal metabolites found in this study may be damaging to abnormally sensitive end-organs, such as the glomerular and retinal capillaries. On the other hand, it is possible that recurrent stressful situations, such as hypoglycemia and/or acidosis, occurring during the course of the disease, result in periods of adrenal cortical hyperactivity, and it is these intermittent spikes which may trigger sensitive capillary end-organs. Finally, it is suggested that adrenal products, presently unknown, may be involved in the development of diabetic vascular disease, but that these products are not measured by the methods we have employed. It is obvious that these are all speculative considerations which do not lend themselves easily to experimental verification.

SUMMARY AND CONCLUSIONS

1. The plasma 17-hydroxycorticosteroids, the urinary excretion of total 17-hydroxycorticosteroids and of the identifiable and individual urinary 17-ketosteroids, as well as the response of these substances to exogenously administered ACTH have been studied in diabetic patients with and without capillary vascular disease.

2. Results of this investigation indicate that there is no evidence of adrenal cortical hyperfunction in patients with uncomplicated diabetes, diabetic retinopathy and nephropathy.

SUMMARIO IN INTERLINGUA

Le Rolo del Cortice Adrenal in Retinopathia e Nephropathia Diabetic

1. Le 17-hydroxycorticosteroides del plasma, le excretion urinari de 17-hydroxycorticosteroides total e del identificabile 17-cetosteroides urinari individual, como etiam le responsa de iste substantias a administrationes de ACTH exogene esseva studiate in patientes diabetic con e sin morbo de vasos capillar.

2. Le resultatos de iste investigation indica que il ha nulle prova de hyperfunction adreno-cortical in patientes con noncomplicate diabete o retinopathia o nephropathia diabetic.

ACKNOWLEDGMENT

This study was supported by a research grant (P.H.S. No. A-697[2]) from the National Institutes of Health of the United States Public Health Service, Washington, D.C.

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DISCUSSION

GEORGE J. HAMWI, M.D., (*Columbus, Ohio*): I want to take this opportunity to congratulate Dr. Rifkin and Dr. Lieberman on a very beautiful and very thorough paper on the study using technics now available.

Since we were one of the earlier groups involved in performing adrenalectomy in attempting to ameliorate the degenerative vascular complications of diabetes mellitus, and since we had thought for awhile that we were able to produce vascular lesions by the administration of these adrenal steroids, I feel compelled to make a comment. I do agree with Dr. Rifkin in everything that has been presented. I think that the longer we work in this particular area at least with the technics available today, we can find no consistent abnormality in the steroids that we are capable of measuring today.There are deviations with hypoglycemia, or acidosis, and these cannot be evaluated.

The one thing that disturbs all of us is the fact that these degenerative vascular complications are really the result of unknown influences working over a long period of time, and a static determination of any substance at one particular period may be wholly inadequate for interpretation.

Probably the benefits obtained in diabetic retinopathy after adrenalectomy or hypophysectomy are the result of alterations in blood pressure and vascular responsiveness, rather than directly due to the hormonal changes.

JOSEPH L. IZZO, M.D., (*Rochester, New York*): I would like to compliment Drs. Rifkin and Lieberman on their very fine piece of work. However, despite the rather impressive evidence they have presented, I am not yet convinced that the adrenal cortex does not play a role in the vascular lesions of diabetes. We have also been interested in the role of the adrenal cortex in diabetes and as part of our studies have followed the daily excretion of total reducing corticosteroids and 17-ketosteroids in selected cases on the Metabolism Ward under various experimental regimens. One of the things which we have noticed is that while the average or mean values of urinary corticosteroids may be similar to that of normals yet if one observes the pattern of excretion for several days one is impressed by the variability in intra- as well as inter- individual values. In other words, the range of excretion of corticosteroids seems to be somewhat greater than that of normals. This may explain why some investigators have reported values the same as normal while others have reported values less than or greater than normal. We agree with Dr. Rifkin that the average excretion of corticosteroids in diabetics with retinopathy is not different from those without retinopathy.

Finally, it should be pointed out that the present authors have examined mainly the 17-ketosteroid metabolites. As you know, this comprises only one fraction of the steroid metabolites. I should like to ask Dr. Rifkin if he has any information on the pattern of

excretion of the alpha-ketolic or the glycol metabolites in the urines of diabetics.

DR. RIFKIN: I should like to thank both Dr. Hamwi and Dr. Izzo for their kind comments. We have had little personal experience in the use of hypophysectomy and bilateral adrenalectomy for the alleviation of diabetic capillary vascular disease. Two of the patients included in this afternoon's presentation have been under the care of Dr. Irving Graef and were submitted to bilateral adrenalectomy. He informs me that one patient has had marked improvement in retinopathy while the other has shown no change since operation. We have, however, subjected three of our patients to intensive cobalt irradiation directed to the pituitary gland, but thus far have been unable to produce any clinical or metabolic changes.

I suspect that the variability of excretion patterns that Dr. Izzo has noted might be expected in diabetic patients when subjected to various stress reactions. We are aware that there are other steroidal metabolites which we have not yet measured, but we hope to have an opportunity to do this in the near future.

The Purpose of Research

Bacon asserted that it had not yet, in his time, been recognized that the true aim of all science is "to endow the condition and life of man with new powers or works" or "to extend more widely the limits of the power and greatness of man." Bacon, however, does not take the position that these are the sole purposes of research. Truth for the sake of truth is by no means excluded. Indeed, by pursuing truth for the sake of the power with which it endows mankind, one also arrives at true knowledge, since without knowledge there is no power. Whether one seeks truth first and utility second, or vice versa, is in fact, the same, since "works themselves are of greater value as pledges of truth than as contributing to the comforts of life." Bacon must be credited with emphasizing the fact that knowledge should proceed in the consciousness that the growth of knowledge is the way to increased power over man's environment.

The prescience of one great intellect perceived, as long as three centuries ago, that the role of science and invention and of technologic education must be not only to search for truth but also to direct the fruits of science in the interests of the community and mankind.

By M. H. Trytten
From *Science,* Vol. 126, No. 3262, July 5, 1957.

Fatal Acute Myocardial Infarction

The data obtained from autopsy series by the Washington University group confirm some widely held views regarding acute myocardial infarction but do not confirm others. The facts regarding the incidence of acute myocardial infarction among 17,000 autopsies that are the most interesting are: (1) the discovery that a change has occurred in the relative incidence of the disease in white individuals of the two sexes from 2:1 (males: females) before 1940 to 1:1 since 1940; (2) the discovery that the incidence in older white women has increased more since 1940 than in other age-sex-groups; (3) the discovery that the difference between the incidence in Negroes and whites is increasing rather than decreasing; (4) the discovery that diabetes mellitus did not have a significant effect on the incidence in Negroes although diabetes mellitus unquestionably has a profound effect on the incidence of acute myocardial infarction among white individuals.

Many investigators have noted a close association between diabetes mellitus and coronary heart disease. The Washington University group found that acute myocardial infarction was four times more common among autopsied white diabetic patients than among autopsied white nondiabetic patients. However, among Negro diabetic patients, acute myocardial infarction was not significantly more common than among nondiabetic patients. Thus a Negro diabetic patient is even less likely to die of myocardial infarction than a nondiabetic white patient. It is interesting to note that diabetic patients with acute myocardial infarction did not die at an earlier age than nondiabetic patients.

From "Fatal Acute Myocardial Infarction: Sex, Race, Diabetes and Other Factors," by Wilbur A. Thomas, M.D., in *Nutrition Reviews*: Vol. 15, No. 4, April 1957, pp. 97-101.

Pathogenesis of Glycogen Infiltration of the Pancreas in Diabetes

Sydney S. Lazarus, M.D., M.Sc. (Med.), Bruno W. Volk, M.D., New York City

Vacuolization or hydropic degeneration of the *B* cells of the islets and of the ductular epithelium is one of the few characteristic morphologic alterations of the pancreas which may be found in both human diabetes mellitus and in experimental diabetes in animals.[1] Ever since the discovery of this lesion in 1911 in the pancreatic *B* cells of patients with severe diabetes,[2] confusion has existed as to its pathogenesis and significance. Experimentally similar lesions were produced in the pancreas of dogs and cats rendered diabetic by partial pancreatectomy.[3, 4] Since with the technics then available it was not possible to demonstrate fat or glycogen or other material in the vacuolated cells, this lesion was interpreted as being degenerative in character and due to imbibition of fluid. The hypothesis was advanced that during the development of diabetes the *B* cells are stimulated to increase their insulin output until they become functionally exhausted. Morphologically it was thought to be evidenced initially by *B* cell degranulation, followed by hydropic degeneration. This was stated to be "the only proved example of anatomical breakdown of cells due to overstimulation of an internal secretory function.[4] It was also noted that vacuolization is also present in ductular epithelium in the end stages of severe diabetes. Since an endocrine function could not be attributed to the duct epithelium, this finding was integrated with the previous hypothesis as representing the "exhaustion of a proliferative function."[4]

Most subsequent workers[5] have accepted the hypothesis that functional overstrain of the *B* cells results in hydropic degeneration. Although many authors noted vacuolization of the duct epithelium, very little attention was paid to its theoretical implications. It is generally assumed that *B* cell vacuolization precedes that observed in the ductules.[6] On the other hand, in some dogs treated with anterior pituitary extracts the presence of vacuolization has been reported in the duct epithelium in the absence of hydropic change in the *B* cells.[7] A study of the time sequence of morphologic change in the pancreas of dogs, made diabetic by crude anterior pituitary extracts, also showed initial B cell degranulation followed by hydropic change.[8] This evidence was employed to support the exhaustion theory of the pathogenesis of hydropic degeneration. The vacuolization of duct epithelium, which was also found, was treated as a more or less incidental, unexplained, and concomitant observation.

It was not until 1951 that hydropic degeneration in the pancreas was shown to be due to the presence of glycogen within the ductular epithelium and *B* cells which is usually removed from these cells during the processing of the tissues.[9] More recently it was demonstrated that glycogen infiltration of duct epithelium occurs in cortisone-treated rabbits prior to and in the absence of it in the *B* cells.[10, 11] Similar results were also obtained in dogs treated with purified anterior pituitary growth hormone.[12] These observations appeared to differ from the hypothesis that hydropic change is due to functional overstrain of the *B* cells, and were thought to support the idea that pancreatic glycogen deposition does not differ pathogenetically from that occurring in other organs in diabetes.[9, 10, 11, 12] The present study was made to clarify further the pathogenesis and significance of this lesion, and to determine whether ductular glycogen deposition precedes that in the *B* cells in other forms of experimentally induced diabetes.

MATERIAL AND METHODS

The study was carried out on twenty-nine mongrel dogs of either sex, weighing between 12 and 15 kg. The animals were divided into four groups. The first group of seven animals was subjected to 85 per cent partial pancreatectomy only. A second series of sixteen dogs likewise underwent 85 per cent partial pancreatectomy. However, after a recovery period of one week each dog received 5 to 10 mg./kg. of cortisone acetate* daily in-

Presented at the Seventeenth Annual Meeting of the American Diabetes Association in New York City on June 1, 1957.

From the Isaac Albert Research Institute of the Jewish Chronic Disease Hospital and the Department of Pathology, Albert Einstein College of Medicine, New York, N. Y.

* Cortisone acetate was supplied through the courtesy of Dr. C. J. O'Donovan of the Upjohn Company, Kalamazoo, Michigan.

tramuscularly for periods of up to thirty-five days. Two additional dogs (Group 3) were treated similarly, except that, after thirty-five and forty-nine days respectively on 8 mg./kg. of cortisone acetate intramuscularly daily, they were given subcutaneously 3 mg./kg. of growth hormone* daily for an additional seven and nineteen days. Two each of four other dogs (Group 4) received subcutaneously 3 mg./kg. of growth hormone for fourteen or twenty-one days, starting on the seventh day after partial pancreatectomy. The various groups of animals utilized for the present experiments are summarized in table 1. Specimens from pancreas removed at surgery served as normal controls.

All animals were maintained on a high carbohydrate diet, consisting of mashed potatoes, meat, meat gravy, and instead of drinking water 5 per cent glucose solution ad libitum. The animals were sacrificed by overdosage with intravenous sodium nembutal at various time intervals, and autopsies were performed immediately after death. Tissues from the pancreatic remnants were fixed in Zenker formol solution (20 per cent) and embedded in paraffin. Sections were stained by the aldehyde fuchsin[13] and a modification of the Masson trichrome method.[14] The periodic acid Schiff method,[15] controlled by diastase digestion, was utilized for the demonstration of glycogen. The blood sugar determinations were carried out by the Nelson modification of the Somogyi method.[16]

* Growth Hormone (Somar-A-List 916 Potency 106.2± 15.9% of Armour Standard) was a gift from the Endocrinology Study Section, National Institutes of Health.

RESULTS

It was confirmed that in sections of normal dog pancreas, stained by the trichrome method, occasional small well-defined vacuoles are present in the epithelial cells of the intercalated and interlobar ducts (figures 1 and 2). With the periodic acid Schiff technic small deposits of glycogen, corresponding to the cytoplasmic vacuoles, are demonstrated in these epithelial cells. However, no glycogen or vacuolization is observed in the B cells.

Thirteen of the dogs, subjected to partial pancreatectomy with or without cortisone administration, developed diabetes in contrast to the others which did not. On the other hand, all animals of both groups receiving growth hormone became severely diabetic.

Two dogs, which did not develop fasting hyperglycemia, displayed moderate *B* cell degranulation. In general, however, nondiabetic dogs showed no alteration in pancreatic morphology except for considerable fibrosis due to surgical intervention. Six dogs developed mild diabetes, lasting from six to forty-one days with blood sugar level varying from 140 to 200 mg. per cent. These dogs showed moderate or marked *B* cell degranulation as well as increase in duct epithelial vacuolization with increased amounts of intracytoplasmic glycogen deposition. However, no B cell vacuolization or glycogen was found. Animals who were severely diabetic for two or three days also showed accentuated vacuolization and

TABLE 1

Summary of experiments

Number of Dogs	Operation	Medication	Dosage	Duration of Medication	Number of diabetic dogs
7	Partial pancreatectomy	None		7-35 days	4
16	Partial pancreatectomy	Cortisone* acetate	5-10 mg./kg. daily	7-42 days	9
2	Partial pancreatectomy	Cortisone* acetate followed by growth hormone	8 mg./kg. daily 3 mg./kg. daily	Dog 49,C.A.† 49 days G.H.‡ 19 days Dog 547,C.A. 35 days G.H. 7 days	2
4	Partial pancreatectomy	Growth hormone*	3 mg./kg. daily	2 weeks (2 dogs) 3 weeks (2 dogs)	4

*Treatment with cortisone acetate and growth hormone was started on the seventh post-operative day.
†C.A.—Cortisone acetate
‡G.H.—Growth hormone.

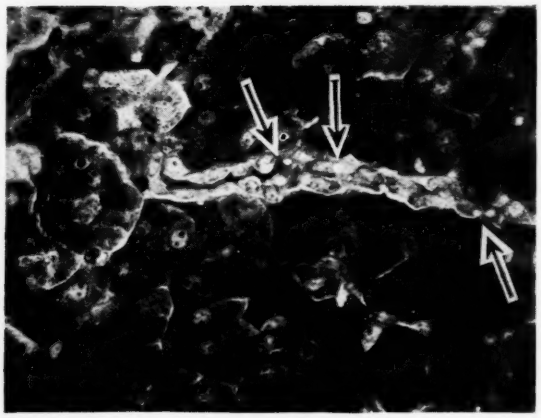

FIG. 1. Pancreas of normal dog, showing an intralobular duct. The arrows point to vacuoles in the cytoplasm of the epithelial cells. Masson Trichrome X-520.

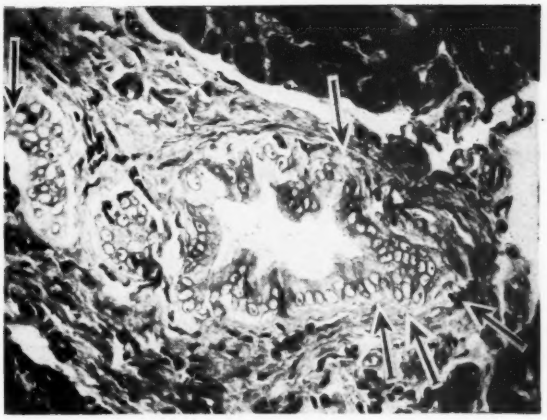

FIG. 2. Pancreas of normal dog, showing an interlobar duct. The epithelial cells contain occasional vacuoles (arrows). Masson Trichrome X-380.

glycogen deposition in the epithelial cells of the intercalated (figure 3) and interlobar ducts (figure 4) but showed no *B* cell glycogen. An animal with severe diabetes for only five days showed increased quantities of duct glycogen and vacuolization of *B* cells due to glycogen deposition. As the duration of diabetes was prolonged these changes became accentuated. In several dogs which were severely diabetic for fifteen to eighteen days, the *B* cells as well as duct epithelium were almost uniformly markedly vacuolated (figure 5), and contained large amounts of glycogen (figure 6). The nuclei of the vacuolated *B* cells appeared normal. The cells were markedly enlarged, and showed thin strands of cytoplasm, radiating from the nucleus to the cell borders. In occasional animals glycogen was found also in acinar cells.

DISCUSSION

The present studies confirm that vacuolization due to glycogen deposition is sometimes present in the pancreatic ductular epithelium of the normal adult dog. This represents apparently a species difference, since in the

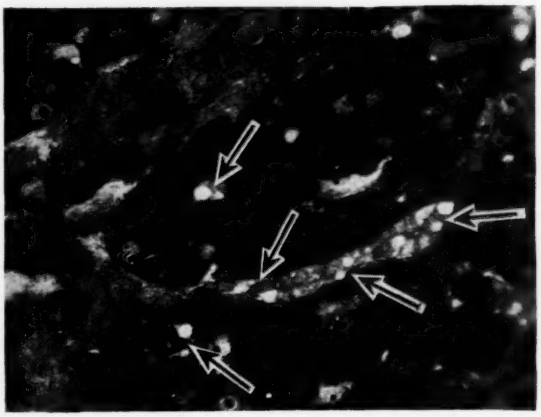

FIG. 3. Pancreas of partially depancreatized dog which after a recovery period of one week received 8 mg./kg. of cortisone acetate daily for thirty-two days and was moderately diabetic for the last six days with a maximum blood sugar of 220 mg. per cent. There is increased vacuolization of the epithelium of an intercalated duct and of centro-acinar cells (arrows). No vacuoles are present in the islet cells (left). Masson Trichrome X-520.

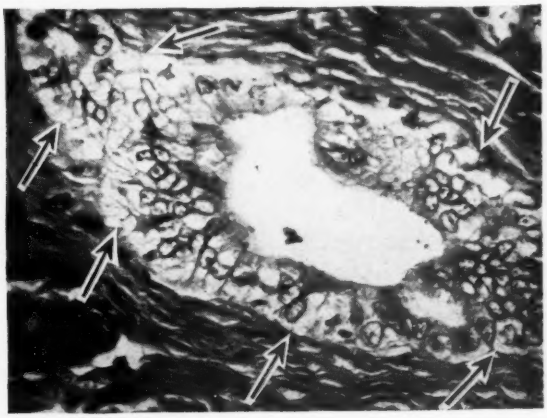

FIG. 4. Pancreas of a partially depancreatized dog which after a recovery period of one week received 8 to 10 mg./kg. of cortisone acetate daily for sixteen days and was severely diabetic for the last seven days with a maximum blood sugar of 350 mg. per cent. The picture demonstrates marked vacuolization of the ductal epithelium (arrows). Masson Trichrome X-500.

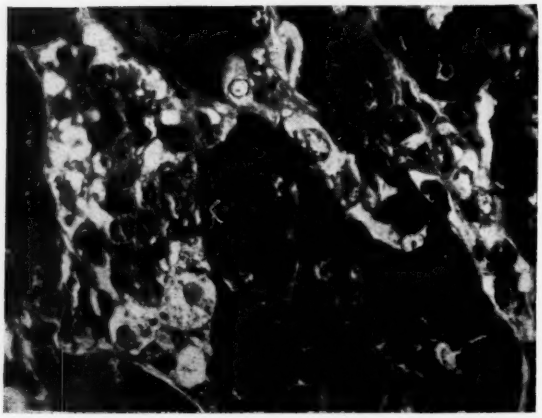

FIG. 5. Pancreas of a partially depancreatized dog which after a recovery period of one week received 3 mg./kg. of growth hormone daily for nineteen days and was severely diabetic for the last sixteen days with a maximum blood sugar of 400 mg. per cent. There is marked vacuolization of an intercalated duct and of the B cells of an adjacent islet. Masson Trichrome X-520.

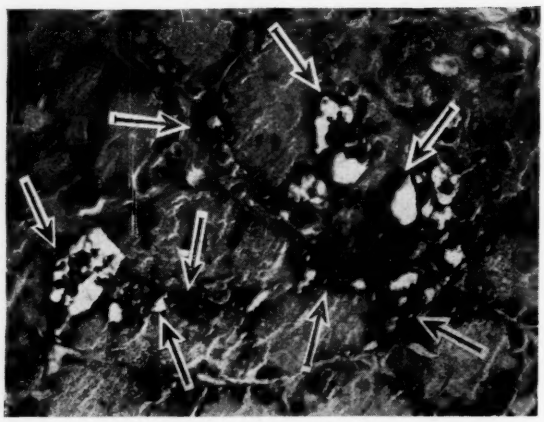

FIG. 6. Pancreas of the same dog as in figure 5, stained by the Periodic Acid Schiff technic. Glycogen (black) is present in the duct epithelium and in the B cells (arrows). X-250.

rabbit[10, 11] and in the guinea pig[17] the normal pancreas does not contain glycogen in the ductular epithelium as it does in the human fetus.[18]

The results of the present experiments show that there is in early stages of surgically and hormonally induced diabetes in dogs increased ductular vacuolization due to glycogen infiltration similar to that observed in the diabetes produced by cortisone in rabbits[10, 11] and by growth hormone in dogs.[12] Furthermore, the increase in glycogen content of the pancreas is concomitant with increase in severity and duration of diabetes. Glycogen appears in *B* cells only after a comparatively long period of diabetes, or with marked severity of diabetes for shorter periods. Moreover, since the ductular epithelium of the dog may normally contain glycogen, and since additional amounts of this material are deposited in the ductules before it is seen in the *B* cells, its appearance in the diabetic pancreas should be considered a quantitative rather than a qualitative change.

It may be assumed from these experiments and those reported previously that the prior deposition of glycogen in duct epithelium is a general phenomenon for various types of diabetes. Since obviously no stimulation of ductular secretory function can be postulated, these observations are in strong opposition to the concept that hydropic degeneration is a histologic expression of a degenerative process, resulting from functional exhaustion. The predominance of duct glycogen deposition over that in *B* cells also makes very unlikely the concept that a peculiarity of *B* cell metabolism related to insulinogenesis is the pathogenetic mechanism. Moreover, it has been previously shown that with regression of experimental diabetes *B* cell regranulation precedes disappearance of glycogen infiltration.[11] This seems to indicate that these two morphologic changes may be independent effects of hyperglycemia and not sequentially related. It appears, therefore, more logical to consider the pancreatic glycogen infiltration as being merely one facet of the ubiquitously increased cellular glycogen deposits which are known to occur in diabetes and are similar to those observed in renal tubular epithelium and in muscle. It is interesting in this connection that glycogen infiltration of renal tubular epithelium was considered a degenerative process until Ehrlich demonstrated that the vacuoles contain glycogen.[19] Renal tubular glycogen deposits are considered at the present time a reversible metabolic phenomenon which is an expression of a high urinary glucose concentration.[20] Furthermore, the increased cardiac glycogen observed in both human and experimental diabetes is accepted as a mere reflection of the elevated blood sugar concentration.[21] Most probably similar mechanisms account for the pancreatic glycogen deposition. The explanation for the initial appearance of glycogen in duct epithelium is not known. However, it has been suggested[11] that it may be related to increased glucose concentration in the external pancreatic secretion in the diabetic animal.[22]

This change in viewpoint, that *B* cell vacuolization rather than being a degenerative lesion due to exhaustion is merely the result of glycogen infiltration as a conse-

quence of hyperglycemia, may have further implications. In other situations glycogen may accumulate within cells without causing their destruction. This is exemplified in von Gierke's disease and in diabetes itself in the renal tubular epithelium. Furthermore, the most marked hydropic change has been produced by adrenal cortical hormone in the rabbit[23] and guinea pig[24] without associated *B* cell destruction or the development of permanent diabetes. Also hydropic degeneration of one years' duration in the pancreas of alloxan diabetic rabbits was not associated with *B* cell destruction.[25] It, therefore, seems that the concept which suggests that glycogen infiltration in the *B* cells is part of the pathogenetic mechanism which leads to their destruction and to permanent experimental diabetes may have to be revised. However, at the present time no alternative explanation is available.

SUMMARY

A study was conducted on the sequential development of hydropic degeneration (glycogen infiltration) in the pancreas of partially depancreatized dogs and in similar animals who received in addition cortisone and/or growth hormone after the seventh post-operative day for various time intervals.

In conformity with previous observations glycogen vacuolization was sometimes normally present in pancreatic duct epithelium. Early in the development of diabetes regardless of the mechanism of induction there was increased vacuolization and glycogen infiltration of the ductular epithelium. On the other hand, glycogen was found in the *B* cells as a comparatively late phenomenon. With increasing severity of diabetes the time required for its appearance in these cells was shortened. These findings together with those previously reported indicate that the prior deposition of glycogen in pancreatic duct epithelium is a general phenomenon for experimentally induced diabetes. These results suggest that the functional exhaustion hypothesis of the pathogenesis of hydropic degeneration in the *B* cells be revised. These lesions should not be considered a degenerative process but rather merely one facet of the generalized increased glycogen deposits seen in the diabetic state similar to those found in renal epithelium and in muscle.

SUMMARIO IN INTERLINGUA

Pathogenese de Degeneration Hydropic (Infiltration de Glycogeno) in le Pancreas Diabetic

Esseva effectuate un studio del disveloppamento sequential de degeneration hydropic (infiltration de glycogeno) in le pancreas de partialmente dispancreatisate canes, incluse un serie tractate additionalmente con cortisona e/o hormon de crescentia a partir del septime die post le operation durante varie periodos de tempore.

De accordo con previe observationes, vacuolisation de glycogeno esseva presente a grados normal in certe casos in le epithelio del ducto pancreatic. Durante le prime stadios del diabete, sin reguardo a qual mechanismo habeva essite usate pro inducer lo, augmento de vacuolisation e de infiltration de glycogeno in le epithelio del ducto esseva presente. Del altere latere, le constatation de glycogeno in le cellulas B esseva un phenomeno comparativemente tardive. Le intervallo de tempore requirite ante su apparition in iste cellulas esseva reducite con plus alte grados de severitate del diabete. Iste constatationes, insimul con illos previemente reportate, indica que le deposition anterior de glycogeno in le epithelio del ducto pancreatic es un phenomeno general in diabete de induction experimental. Le resultatos suggere que le hypothese del exhaustion functional como factor pathogenetic in degeneration hydropic in le cellulas B pote esser revisionate. Le lesiones non deberea esser considerate como un processo degeneratori sed plus tosto como solmente un de plure aspectos del generalisate augmento del deposition de glycogeno que es vidite in le stato diabetic e que es simile al deposition trovate in le epithelio renal e in le histos muscular.

ACKNOWLEDGMENT

The authors wish to acknowledge the technical assistance of Miss Theodora Green, Mr. Maurice Gordon and Miss Josephine McLeod. Mr. Herbert A. Fischler prepared the photomicrographs.

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DISCUSSION

MILTON M. HESKEL, M.D., (*Philadelphia*): I believe Toreson reported that when small doses of insulin were used, in spite of continued hyperglycemia, interductile glycogen deposits were no longer demonstrable. Did Dr. Lazarus use insulin in his experimental animals?

W. S. HARTROFT, M.D., PH.D., (*St. Louis*): I think Dr. Lazarus and Dr. Volk are to be complimented on their complete systematic study of a phenomenon that has only been observed incidentally in most reports of experimental diabetes.

Their evidence does strongly support their conclusion, that vacuolation does not represent an "oversecretory" phenomenon.

I think, however, their point concerning vacuolation in ducts does not entirely prove the above conclusion, because under certain types of stress, one can observe development of granulation in small ductules near islets. Ductular cell granulation in certain conditions suggests that the cells may undergo metaplasia to secreting beta cells. The possibility therefore exists that vacuolated ductule cells could still represent the next stage — i.e., that of "oversecretion." I hope my colleague, Dr. Lacy, will apply his electronmicrographic methods to study this.

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): If I understand this, the authors have made a distinction between the functional significance of degranulation and of glycogen deposition or hydropic change. To repeat, they view degranulation as a manifestation of functional exhaustion, but hydropic degeneration as a local site of glycogen synthesis. Is that a correct understanding?

IRVING GRAEF, M.D., (*New York City*): Have the authors done any lipid stains or other histochemical studies on their material? If so, what were their results?

SYDNEY S. LAZARUS, M.D., (*New York City*): The first question concerned itself with the experiments of Duff and Toreson, in which small doses of insulin administered to severely alloxan diabetic rabbits were claimed to have prevented or reversed hydropic degeneration, despite the maintenance of hyperglycemia. This they adduced as evidence of the fact that hydropic degeneration resulted from a lack of insulin, rather than from hyperglycemia.

There is, however, a lot of other evidence which is in conflict with this conclusion. To cite but one example, other workers have shown that glucose administered into the pancreatic artery, causes hydropic degeneration of *B* cells concomitantly with marked peripheral hypoglycemia, indicating an increased amount of circulating insulin. This supports the view that hydropic change is a resultant of hyperglycemia.

The question raised by Dr. Lukens concerning the separation of degranulation from hydropic change is quite valid. It has been seen morphologically in regression experiments, that as the blood sugar declines, hydropic change of B cells may be seen together with granulations within the residual intact cytoplasm, indicating that these two phenomena may be dissociated.

The problem of fat infiltration in the pancreas was not studied by us. However, in some instances, I believe in Toronto, an increase in lipids has been demonstrated in the pancreas in diabetic animals.

Acute Development of Diabetic Cataracts and Their Reversal

A Case Report

Hans W. Neuberg, M.D., John H. Griscom, M.D., and Robert P. Burns, M.D., New York City

The acute development of cataracts and their complete reversal in the diabetic patient occur quite rarely. A thorough search of the literature has disclosed only six documented case reports[1-5] in which both of these events were observed (table 1). There are four more diabetic patients who had cataracts of undetermined duration when they were first seen, and which then cleared, coincident with regulation of their diabetes (table 2).[6-8] Because the rapid appearance and disappearance of such cataracts has been reported so infrequently, the following case is recorded in which the abrupt development and rapid and complete reversal of cataracts were observed upon initiation of insulin therapy and in the absence of severe dehydration or acidosis.

CASE REPORT

A.G., Presbyterian Hospital unit No. 289973.

The patient, a fifty-two-year-old colored male cook, was first seen at the Presbyterian Hospital in September, 1956, complaining of marked weakness and weight loss of about six months' duration. His past history revealed many years of ethanolic excess associated with attacks of nausea, vomiting and epigastric pain radiating to the back, but these symptoms had disappeared abruptly eight years prior to admission, when he ceased drinking. There was in addition a history of a penile lesion in 1923, which had been treated with "hip shots and pills" without subsequent symptoms that could be related to syphilis. His personal and family history were not relevant, and a family history of diabetes mellitus was denied by name or symptom.

He had been apparently in good health until six months prior to admission, at which time he began to note progressive muscular weakness, numbness and paresthesias of the extremities, lethargy, easy fatigability and decreased libido. Shortly afterwards, diarrhea consisting of one or two daily loose, oily stools, developed in association with fatty food intolerance. About two months prior to admission he noted proptosis and swelling of both periorbital regions associated with slight visual blurring, parotid gland enlargement and edema of the feet, along with marked polydipsia, polyphagia and polyuria, and also the progressive loss of thirty-five pounds.

On physical examination: T 100°, pulse sixty-six, respirations sixteen, blood pressure 100/70. He was cachectic with dry hyperkeratotic skin. Marked periorbital edema, chemosis and proptosis (O.D. 29, O.S. 28 by exophthalmometry) were present with a small corneal ulceration O.D. The fundi were well visualized by four observers and noted to be normal. No cataracts were seen, and the media were clear. The parotid glands were massively enlarged (right more than left) with two centimeter swelling of both submandibular glands. The lacrimal glands were normal. There was no generalized lymph node enlargement. The thyroid gland was normal. The heart, lungs and abdomen were normal. There was 1+ bilateral pedal edema. There was no tremor. The deep tendon reflexes were markedly diminished with generalized muscular weakness, as well as atrophy, which was greatest in the proximal muscle groups of arms and legs. Sensation was intact. Cranial nerves were intact except for mild exophthalmic ophthalmoplegia.

LABORATORY DATA: *Hemogram*: Hct. 40 per cent, Hgb. 13 gm. per cent, WBC 7,800 (polymorphs 57 per cent, lymphocytes 41 per cent, monocytes 2 per cent). ESR 10 mm./hr., (Westergren). *Urinalysis*: Sp. gr. 1.032, 4+ glycosuria, but negative for acetone and diacetic acid, protein and the microscopic examination was negative. *Mazzini*: negative. *Stools*: negative for occult blood, but they were foul, oily and 4+ positive for fatty acid. *Old tuberculin skin test*: positive at 1:1,000. *Electrocardiogram*: normal. *Cerebrospinal fluid*: dynamics normal, no cells, protein 28 mg. per cent, Kolmer: negative. *Blood chemistries*: Fasting blood sugar 165 mg. per cent, and two hours after breakfast it was 334 mg. per cent. Serum sodium 138 mEq./L. Potassium 4.5 mEq./L. CO_2 25 mEq./L. Serum albumin 2.6 gm. per cent, serum globulin 3.2 gm. per cent, (filter paper electrophoresis). Alkaline phosphatase 2.7 Bodansky units. Bromsulfalein retention 2 per cent at thirty minutes. Serum cholesterol 126 and 142 mg. per cent. *Cephalin flocculation and thymol turbidity*: negative. *Prothrombin time*: normal. *Serum amylase*: 52 Meyer and Killian units. *Serum carotene*: none. *Serum calcium*: 8.1, 9.3 and 10.5 mg. per cent. *Serum phosphorus*: 3.1 and 4.7 mg. per cent. *Protein bound iodine*: 4.4 mcgm. per cent. *Radioactive iodine uptake of thyroid gland*: 11 per cent and 28 per cent in twenty-four hours on two occasions. *Basal metabolic rate*: —15 per cent and —11 per cent. *X rays*: Gallbladder series, normal, with questionable widening of the common duct. Gastrointestinal and small intestinal series showed diffuse pancreatic calcifications but no other abnormalities, and specifically no sprue pat-

Presented before the Clinical Society of the New York Diabetes Association, May 25, 1957.

From the Departments of Medicine and Ophthalmology, Columbia University College of Physicians and Surgeons, the Presbyterian Hospital, and the Institute of Ophthalmology, New York, New York.

TABLE 1

Development of acute diabetic cataracts and their reversal
(Review of literature)

Author	Patient	Duration of diabetes	Cataracts	
			Onset	Clearing
Neuburger, 1893[1]	17-yr. girl	2 years	11 days to full development. No information about therapy at onset, then dietary control.	3-6 weeks, despite very poor dietary control of diabetes.
Alt, 1906[2]	28-yr. woman	?, but short	6 hours to complete loss of vision.	Began clearing in 1 week, with dietary control; complete clearing in 3 weeks. Died 14 months later in diabetic coma.
Braun, 1935[3]	16-yr. girl	not known	6 days to marked opacification, after insulin treatment.	Began clearing in 4 days, with insulin. Complete clearing in 16 days on 120 units of insulin.
Lawrence, 1946[4]	39-yr. man	2 months	After 16 days of insulin slow formation of bilateral partial cataracts of rosette type.	Lasted 4 days, clearing in 4 more days on insulin.
Lawrence, 1946[4]	45-yr. man	2 weeks	Onset 3 days after dietary control.	Improving after 25 days, complete clearing on diet in 1 month.
Jackson, 1955[5]	19-yr. girl	3-5 months	Onset within 24 hours, after 4 days of insulin treatment.	Improvement after 19 days; 30 days to complete clearing.

TABLE 2

Reversal of diabetic cataracts
(Review of literature)

Author	Patient	Duration of diabetes	Cataracts	
			Onset	Clearing
Seegen, 1870[6] cited by Nettleship	39-yr. man	6 months	Presenting with cataracts. ? duration. Dietary control.	Improving in 9 days; O.S. completely clear, O.D. only minimal residual opacity. Died 2 years later, "blind."
Seegen, 1870[6] cited by Nettleship	55-yr. woman	6 months	Presenting with cataracts. ? duration.	Dietary control, with "decreased cataracts." Poorly documented.
Tannahill, 1884[7] cited by Nettleship	44-yr. man	1 year	Appeared with bilateral cataracts, left more than right. ? duration.	After 14 days of opium therapy, clearing completely.
Fischer, 1925[8]	17-yr. girl	not known	Appeared with bilateral cataracts. ? duration, and very poorly controlled diabetes, progressing to coma; insulin therapy.	1 month after insulin therapy, clearing completely within 8 days.

tern. X rays of chest, skull, orbits, hands and long bones were within normal limits. Radioactive iodine-labeled neutral fat and fatty acid absorption studies showed marked impairment of absorption of both. Sialograms were normal. *Biopsies of skin and muscle* showed nonspecific myositis, and biopsy of the parotid gland showed normal tissue.

COURSE IN THE HOSPITAL: The patient was begun on a regimen including standard insulin and a diet of 3,000 calories, low in fat, high in protein and including 200 gm. of carbohydrate. During the first thirty-six hours he received a total of 75 units of insulin, which precipitated a transient, mild episode of hypoglycemia. The ultimate control of his diabetes, however, was quite difficult. His constant polyphagia necessitated an increase in total calories to 3,500-4,000 daily with 240 to 260 gm. of carbohydrate. On his third hospital day, depot insulins were begun, and increasing quantities were required over the next forty days, with the dosage finally reaching 140 to 160 units daily. At length, adequate control was established with 150 units of NPH insulin and 20 units of standard insulin each morning, with a 3,000-calorie diet containing 150 gm. of carbohydrate. His dietary demand seemed to subside concomitantly with control of his diarrhea by the addition of pancreatin, and establishment of adequate insulin dosage.

Of special interest was the abrupt onset of marked diminution in vision forty hours after the initiation of insulin therapy and four hours after a transient, mild episode of hypoglycemia. At this time he was found to have developed large club-shaped, stellate radiating opacities of both lenses with central scoto-

FIGURE 2

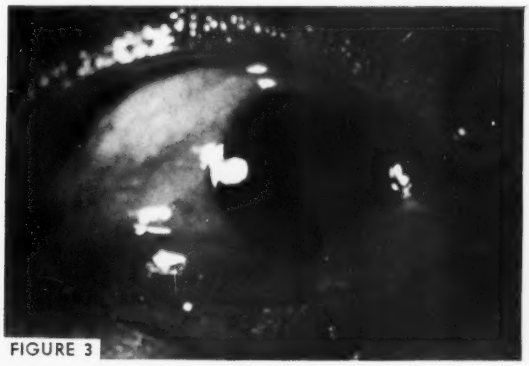

FIGURE 3

FIGURE 4

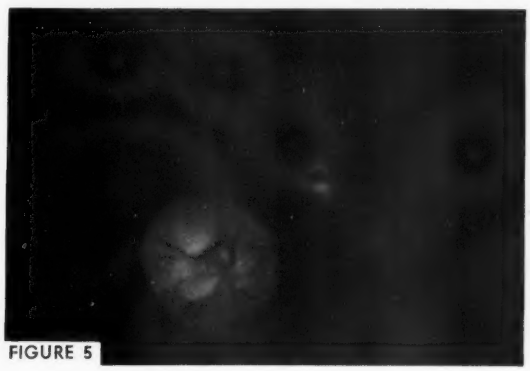

FIGURE 5

FIG. 2. On hospital admission Sept. 13, 1956, the eyegrounds were visualized and considered normal by four observers who were searching for signs of papilledema or diabetic retinopathy, without mydriasis.

Subsequently, further decrease in vision and pain in the right eye developed. First ophthalmologic examination on Sept. 15, 1956, showed marked exophthalmos with right chemosis and an exposure corneal ulcer. Extra-ocular movements were limited. Visual fields showed a central scotoma. The visual acuity was 20/400 in the right eye and 20/100 in the left eye. There were dense lens opacities which prevented a view of the red reflex until the pupils were dilated. On maximal pupillary dilatation, the cataract was seen to lie in the deeper cortical layers, and was stellate with branching and club-shaped ends. On slit-lamp examination, the lens opacities appeared to be clefts in the lens substance, near and in the sutural areas, composed of fluid of a clear and granular nature. With the pupils dilated, the periphery of the fundus was visible, and the lens appeared normal except for the stellate opacities.

FIG. 3. The corneal ulcer cleared with bandaging, but the vision continued to deteriorate. By September 19, the lenses were completely clouded in the previously clear areas with a bluish-white translucent opacity which prevented any view of fundus details. This observation was confirmed at the weekly Institute of Ophthalmology Grand Rounds. On September 21, the corneal ulcer recurred in the right eye and the lenses were milky white, with the appearance of a mature cataract. (Note: only right eye shown—appearance of left eye identical.)

FIGS. 4 & 5. Improvement then occurred: By September 21 extra-ocular movements were normal; the central scotomata were smaller on September 24; the chemosis was gone by September 25; and clearing of the lens opacities was noted first on September 26. The cataracts disappeared in reverse order of their appearance, with the milky white cortical opacities disappearing rapidly. The deeper stellate cleft-like opacities persisted until Dec. 6, 1956. Exophthalmos persisted until November at about the same level, then had decreased 4 mm. in the right eye and 3 mm. in the left by December 6. (Note: only right eye shown—appearance of left eye identical.)

The hyperopia often seen in recently treated diabetics was seen in this patient. Corrected visual acuity had returned to normal 20/20 levels by October 26, but hyperopia persisted until December 6. By Feb. 4, 1957, there was no hyperopia and uncorrected vision was 20/20.

mata, after four observers had previously described clear lenses with clear visualization of the eyegrounds as mentioned above. The cataracts resolved with increasing return of vision, and by the time of discharge, seven and one-half weeks after admission, only a few water clefts remained as evidence of the previous cataracts. During the past five months he has been followed in the metabolism and ophthalmology clinics, where he has maintained his improvement. Following one bout of hypoglycemic coma his regimen now consists of pancreatin, 100 units of NPH insulin each morning and a 150-gm. carbohydrate diet. Vision is normal and there is no longer evidence of cataracts (figs. 1-5).

Extensive diagnostic studies failed to reveal the cause of exophthalmos, chemosis or parotid swelling, which gradually disappeared by the time of discharge. Increasing attention was focused on the gastrointestinal tract, with the finding of diffuse small pancreatic calcifications and the subsequent demonstration of interference with absorption of fat, zero blood carotene, fatty stools and hypo-albuminemia. Serum calcium, originally low normal, became normal during the hospital stay. The patient's diarrhea and fatty food intolerance subsided following administration of pancreatin. Over the next two months, the patient's sense of well-being returned and his weight increased from 117 lb. to 155 lb. with control of diabetes and steatorrhea. His strength returned coincidentally.

DISCUSSION

The rapid appearance and relatively rapid disappearance of lenticular opacities in this patient are quite similar to the events described in the six previously published cases[1-5] (table 1). The patient's cataracts were classically "diabetic," in that they were first seen as lamellar stellate clefts in the lens, progressed to total opacity of bluish-white color, and then regressed rapidly in the reverse order of their onset.

The simultaneous presence of exocrine pancreatic insufficiency, owing to chronic pancreatitis, complicates etiologic delineation, but acute cataracts have not been described in this disorder. The relationship of the parotid swelling to the other disease processes will be discussed below. No explanation can be offered for occurrence and subsidence of exophthalmos.

Etiology of acute diabetic cataracts: The etiology of diabetic cataracts has recently been reviewed extensively by Patterson.[9] While there are many experimental methods of producing cataracts[10] only a few are relevant to this discussion. The following two theories have been proposed to explain the acute development of cataracts:

1. Protein denaturation. While the sudden onset of cataracts could well be explained on the basis of structural changes in the lens protein, it would seem highly unlikely that these changes can be reversed completely in the relatively brief time described here. Available data suggest that the relative proportions rather than the nature of the lens proteins themselves are affected in "chronic" cataractous changes.[11] The proteins of *acute*

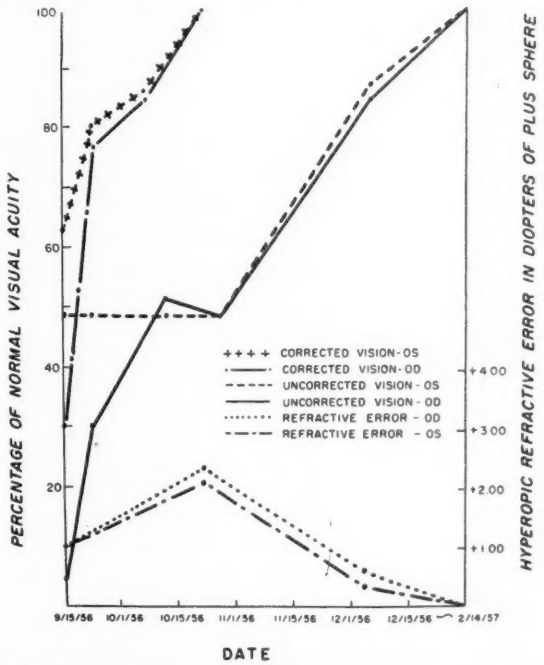

FIG. 1. Quantitative ophthalmological measurements of patient's course.

cataracts have apparently not been studied. However, if lens protein changes play a part, they may well be related to

2. Changes in lenticular glucose metabolism and osmotic changes, with disturbances of the finely balanced protein-fluid relationships, and sequestration of fluid droplets. Thus, sudden changes in refraction in diabetic patients are believed to be partly the result of intralenticular osmotic changes brought about by the rapid alteration of the sugar content of the blood and perhaps of the aqueous and vitreous content. The sudden appearance of cataracts in the severely dehydrated patient with cholera[12] is probably similarly related to disturbed intralenticular osmotic relationships. However, the acute formation of cataracts has not been described in diabetic acidosis with its severe disturbance of hydration and electrolytes, although, when specifically sought for, lenticular changes such as dark lines can be seen, with reversal on rehydration.[13]

In order to attempt to assess the factors important in the development of cataracts in the diabetic, it has to be understood that the lens itself does not have a blood supply, but is dependent for its water, electrolyte and metabolic exchange on the metabolic activity of the lens

capsule and the composition of the surrounding aqueous and vitreous medium. The composition of the aqueous is, in turn, controlled by the activity of the ciliary body. While the metabolism of the lens is obviously an enormously complex and as yet only partially understood phenomenon, it is thought that the bulk of lenticular energy requirement is furnished by glucose. The following facts are known about the transport of glucose from blood to lens, the metabolism of glucose by the lens, and the effect of insulin on these two processes.

Transport of blood glucose to the aqueous by the ciliary body is thought to be a process of diffusion and secretion. In the strict sense of the word, there is no active transport, since glucose content of the aqueous is lower than that of the blood, in part at least, because of the consumption of glucose by the lens and cornea.[14] In the experimental animal, glucose concentration of the aqueous roughly parallels that of blood.[15, 16] Insulin, in doses of less than two units, has been shown to increase glucose transport into the aqueous of the rabbit, while with larger doses (10 units) glucose transport returns to control levels, possibly related to coincident hypoglycemia.[17] The vitreous also contains glucose, in concentrations higher than the aqueous, and probably higher than the lens.[15] It is of some interest that the center of the vitreous contains a higher glucose concentration than either the retinal or the lenticular poles, presumably because of glucose consumption at these poles.[18] There are no data on the effect of insulin on vitreous glucose concentration.

The gradient of glucose between aqueous and lens depends, perhaps to the largest extent, on diffusion. While in vivo, the glucose concentration of the lens is lower than that of the aqueous, glucose concentration of the isolated intact rabbit lens is directly proportional to the glucose concentration of the medium over a wide range of concentration.[19] The isolated *intact* lens of the diabetic rat shows a decreased glucose consumption, which could not be reversed by insulin added to the incubation medium.[20] Also, in the isolated *intact* rabbit lens, no change in intralenticular glucose concentration could be shown with the addition of insulin to the incubation medium.[19] The only preparation in which an insulin effect could be demonstrated was the *decapsulated* rabbit lens,[21] where intralenticular glucose concentration was increased by added insulin. In view of the probable metabolic importance of the capsule, this effect of insulin is therefore difficult to evaluate. It is of some interest, however, that the isolated lens of the diabetic animal which has been previously injected with insulin shows a considerable increase in glucose consumption.[20]

The pertinence of in vitro experiments with added insulin may be questioned, since at this time there is no evidence that there is any insulin in the aqueous medium, and some evidence that it may not be present.[19, 22] However, more experimental data are needed on this point—especially since proteins of equal or larger molecular size can be shown to be present in the aqueous.

While it seems attractive to relate these changes in glucose concentration to intralenticular osmotic changes, there is no experimental evidence bearing directly on this point. Thus, there are no data on experimental hypoosmolarity of the aqueous. Cataracts have been produced by the injection of hypertonic sucrose and saline into animals. It is by inference only that diabetic cataracts can therefore be regarded as "osmotic" cataracts.

That factors other than glucose levels in aqueous and lens can be responsible for experimental cataract formation has been proposed by Patterson.[9] He has found that in fructose fed rats, whose diet was supplemented with other energy-yielding substances (casein and fat), but whose blood sugars were high because of conversion of some fructose to glucose, there is no formation of cataracts. He therefore suggests that hyperglycemia per se is not important in cataract formation, but only the availability of energy-yielding substances. He suggests that in the diabetic animal, despite higher levels of glucose in blood and aqueous, glucose cannot be utilized by the lens, and cataracts develop. If alternate food substances are provided, no cataracts are formed. However, this theory does not account for the fact that in the diabetic animal, speed of cataract formation is roughly proportional to blood glucose levels, and that any factor that lowers blood sugar, be it dietary control, insulin or phlorizin, can delay or prevent cataract formation. On the other hand, Patterson's theory might account for the occurrence of cataracts in the dehydrated patient with cholera, while they do not occur in the severely dehydrated diabetic in acidosis.

Thus, from the experimental data, there arises no obvious explanation of the sudden occurrence of cataracts in diabetic patients. In each of the six previously reported cases[1–5] cataracts occurred very soon after the institution of diabetic control, either with or without insulin. Clearing occurred with continued diabetic control, apparently without any alteration in the therapeutic regimen, both in the published cases and in the one presented here.

Scotomata: Although, in this patient, cataracts and central scotomata were observed to appear and resolve simultaneously, it is generally accepted that cataracts do not cause central scotomata. Macular disease, such as

hemorrhage, edema or vasospasm, can cause central scotomata and occurs in diabetes mellitus. The cataracts obscured the fundi until the central scotomata cleared, but no macular disease was observed prior to the formation of cataracts, and it is thus thought unlikely that macular disease caused the scotomata. A more likely cause of the central scotomata would be optic neuropathy, which is sometimes seen in diabetic patients, often in association with malnutrition. However, since exophthalmos and central scotomata appeared and resolved simultaneously, it is felt that the most likely explanation is that pressure or traction on the optic nerves secondary to acute orbital edema caused ischemia of the optic nerves with resultant central scotomata.

Malnutrition: While there is no evidence that pancreatic exocrine deficiency has ever caused the formation of cataracts, it seems pertinent to review those factors which might have been contributory in this patient. Associated with his steatorrhea there was probably some difficulty in absorption of proteins and lime salts. There has been no report of cataract formation due to diminished fat absorption. In young growing rats, cataract formation has been initiated and potentiated by amino acid deficiency, particularly tryptophan.[23] However, Pirie and Van Heyningen[23] after reviewing the evidence feel that protein deficiency per se is not a real factor in cataract formation, and, actually, in alloxan diabetic animals, protein feeding has increased the rate of cataract formation.[24] Experimentally, cataracts have also been produced by a low calcium/phosphorus ratio in the diet, but only when serum calcium has reached levels associated with tetany.[25] While the factors of malnutrition can, therefore, not be totally dismissed, there is no evidence at this time that they do contribute to the formation of cataracts.

Parotid swelling: Another interesting feature of our patient's illness was the appearance and disappearance of striking parotid swelling. There was no evidence of parotitis on biopsy, and such diseases as Boeck's sarcoid seem to have been ruled out by the studies cited above. Parotid swelling has been described in malnutrition,[26] but there appears to be little valid evidence that parotid swelling is due to diabetes mellitus. Close examination of the literature describing parotid swelling in diabetes mellitus reveals that in one reported and cited case, parotid swelling of unknown nature antedated the development of diabetes by several years.[27] In another series of four cases, parotid swelling did appear during the course of diabetes mellitus. However, no data were given to rule out common causes of parotid swelling, and no follow-up data were given.[28] In one large series of patients with parotid swelling associated with malnutrition, diabetes mellitus was found in 10 per cent of the patients, but documentation of both parotid swelling and diabetes was extremely sketchy, and, as noted, all patients were malnourished.[29] Thus, while the literature suggests that there may be coexistence of diabetes mellitus and parotid swelling, no proof of their causal relationship is present.

SUMMARY

A patient with diabetes mellitus is reported in whom bilateral cataracts developed within forty hours after the initiation of therapy with insulin, and in whom the cataracts disappeared in about seven weeks of continued insulin therapy. Other similar cases in the literature have been reviewed, and possible mechanisms for acute cataract formation have been discussed. The patient reported here exhibited a series of disorders probably bearing no relation to his acute development of cataracts.

SUMMARIO IN INTERLINGUA

Disveloppamento Acute De Cataractas Diabetic E Lor Reversion: Reporto De Un Caso

Es reportate le caso de un patiente con diabete mellite, in qui cataractas bilateral se disveloppava intra quaranta horas post le initiation de therapia a insulina e in qui le cataractas dispareva in circa septe septimanas de continue therapia a insulina. Altere simile casos in le litteratura es passate in revista, e le mechanismos possibile del formation de cataractas es discutite. Le patiente del presente reporto exhibiva un serie de disordines que esseva probabilemente sin relation con su disveloppamento acute de cataractas.

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Applied Physiology

Speaking at the annual dinner of the Cambridge Graduates Club of St. Bartholomew's Hospital, Sir Henry Dale, O.M., G.B.E., F.R.S., explained how it came about that he only spent a short time at his own hospital after graduation. He had already tried his hand at physiologic research when he was offered the post of intern to Dr. Samuel Gee. In making him the offer Dr. Gee told him that when he entered his wards he must forget all his physiology, which was an experimental science; medicine was not—it must be regarded as an empirical art, and that was what Sir Henry would learn. Fortunately, he was then offered a George Henry Lewes studentship in physiologic research, and he has lived to see the day, which he said Dr. Gee had grudgingly admitted might possibly come, when medicine has become an experimental science with physiology as its foundation.

It is strange how much initial resistance there was to the idea of recognizing medicine as a natural science, and indeed the centenary essays that have been contributed in the *British Medical Journal* to commemorate the birth of Sir Victor Horsley have all stressed the great efforts he made to apply physiology both to anatomy and to disease. Sir Geoffrey Jefferson, in paying his tribute, points out that it was Horsley who showed that the anatomy on which a surgeon's training is traditionally based must have "life breathed into it by physiology." Similarly, Sir Arthur McNalty recalls that when Horsley began his work at University College in London much of the anatomy and physiology of the brain was unknown territory and that Horsley and his colleagues were among the first to "burst into the silent sea" of neurophysiology.

It is certainly one of the greatest advances in the principles of the practice of medicine that the imposing of bizarre personalities has been replaced by the application of accurate scientific knowledge and that the golden-headed cane and the placebo have been ousted in favor of specific remedies. On the other hand it should be remembered that every patient has his own personality; it was perhaps because of this that many physicians of latter days gained tremendous success with their patients by the imposition of their own sometimes bizarre personalities.

The successful modern clinician, however, must be a man of many parts. Without relying on any bizarre characteristics with which to impress his patients he must try to combine the scientific approach with the human understanding that every patient expects.

John Lister, M.D., in "By the London Post," *The New England Journal of Medicine,* page 1233, June 27, 1957.

Tentative Explanation of the High Incidence of Diabetes

Richard H. Post, Ph.D., and Priscilla White, M.D.,† Boston*

INTRODUCTION

From the point of view of population genetics and evolution, the high incidence of diabetes mellitus presents a problem. In almost all modern populations it is considered a "common" disease, and it has been well known since ancient times in areas as widely separated as China, India, Palestine and Europe.[1] Nevertheless it has been killing sufficient numbers—prior to the development of modern therapy—at early enough ages (i.e., before the end of the reproductive period) to have been making a constant drain on the frequency of the gene or genes which are assumed to produce it.

Present consideration of this problem rests on conclusions presented in an editorial of DIABETES (*1*:307, 1952), principally that (1) predisposition to diabetes in a great majority of pedigrees is inherited as a simple Mendelian recessive; and that (2) the frequency of the diabetes-producing genotype (which may be termed *dd*) in the United States today is estimated at about 5 per cent, and the disease incidence at about 1 per cent. Thus about one *dd* person in five develops the disease—i.e., penetrance of the genotype is about 20 per cent.

Most genetically determined diseases are rare. Unless favored by selection the incidences of the provocative genes are low multiples of their mutation rates, reflecting the low reproductive rates of the affected persons. Maximum estimates of the mutation rates of human genes are only 6 to 8 per 100,000 per generation.[2] These figures fall far short of the frequency of the gene *d* estimated above at 22,400 per 100,000 (the square root of 5 per cent, which is the estimated frequency of the genotype *dd*).

No explanation of evolutionary change in gene frequency of such magnitude is adequate except that of a very strong force or forces of positive selection.

The presence of such a force is suggested by various published series of data aggregating several thousands on the sizes of families having *Dd* and *dd* parents. In three types of matings—(A) both parents diabetic, (B) one parent, and (C) neither parent diabetic—these data indicate a positive association between *dd* parents and family size, as noted by Steinberg.[3] Unfortunately for the present purpose the data are biased in that all families were selected for having at least one diabetic child, but a correction of this bias (which cannot be made properly from all of the series as published) would tend to increase the apparent association rather than to diminish it. In the following summary (table 1) it is noted that the average number of living offspring from mating types (A), (B) and (C) are respectively 5.3, 5.0 and 4.5.

The association between the number of parents who are diabetic and the number of children they produce has been cited as evidence that higher parity may precipitate diabetes in women who are potentially diabetic.[4] Such induction has also been termed, in effect, 'not yet proven,'[5] and it is clear that the mere fact of association between two variables does not prove that one is cause and the other is effect.

Admittedly the data of table 1 are unsatisfactory for several reasons. They include no matings of *DD* parents. They make no distinction in the parents' sex, so that the relative procreative success of *dd* mothers and fathers cannot be compared. They were collected in part before, in part after the development of insulin therapy. The average sizes of families proceeding from all three types of matings are large. (This may be explained in part, however, by the facts that most families were larger one or two generations ago than today, that many of these families were rural, and that each had at least one child, i.e., there were no childless families.) The shortcomings may be explained largely by the fact that the data were collected to demonstrate genetic phenomena and not differential fertility.

Another large body of data showing association between diabetes and family size which is not included in the table is that of Pyke.[4] He reported that 394 mothers with diabetes onset at forty-five or over, having at least one child surviving its first year, averaged over 3.2 live births per woman, contrasted with an average of slightly

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TABLE 1

Average number of children in families having at least one diabetic child for three types of matings

Author	(A) Both Parents Diabetic			(B) One Parent Diabetic			(C) Neither Parent Diabetic		
	Number of families	Total children	Average size	Number of families	Total children	Average size	Number of families	Total children	Average size
Steinberg & Wilder 1952	22	122	5.6	370	1,990	5.4	1,589	8,253	5.2
von Kries 1953	8	35	4.4	160	663	4.1	1,137	4,877	4.3
Harris 1950	8	43	5.4	109	484	4.4	1,124	4,541	4.0
Thompson & Watson 1952	4	28	7.0	166	937	5.6	828	4,664	5.6
Pincus & White 1934	(2 or 3)			81	380	4.7	440	1,935	4.4
White 1932	2	4	2.0	33	101	3.1	491	1,453	3.0
Totals	44	232		919	4,555		5,609	25,723	
Pooled Averages			5.3			5.0			4.6

under two live births for English and Welsh women having at least one child surviving its first year according to the 1951 census. Smaller bodies of data have also sustained the association[6, 7, 8] and none is known which refutes it. Until the relationship between diabetes and pregnancy is better understood, however, the conclusion that the *dd* genotype increases fertility must be considered suggestive rather than definitive.

If it be true that diabetics of either sex or both are apt to have more children than nondiabetics (considering only those onsets which follow the childbearing years) a selective superiority would be attributable to the homozygous genotype *dd* over the other two genotypes, *Dd* and *DD*. This would be surprising, since it is the heterozygote which has the highest selective value in the few cases which have been described of differential selection among the three genotypes produced by a single gene mutation. Nevertheless, there is no a priori reason to deny the possibility of the recessive homozygote *dd* having selective advantage over both *Dd* and *DD*, with little or no selective difference between them, under normal conditions.

HYPOTHESIS

A possible explanation of the adaptive superiority of persons with the *dd* genotype may be found in the early adolescent growth spurt and sexual maturity of diabetics which has been reported by White,[9] Wagner *et al*[10] and others. Small groups with childhood and adolescent onsets have been shown to be approximately one year younger than expectancy as to height, weight, carpal and metacarpal development, and several manifestations of sexual maturity.

While one is inclined to attribute this rapidity of growth to the effects of the disease, the suggestion has been made that it might be a pleiotropic effect of the *dd* genotype not directly associated with diabetes.

If it could be demonstrated that persons who later in life become diabetic (and thus have the *dd* genotype but no diabetes during adolescence) become sexually mature at significantly younger ages than normal, it would not be unreasonable to suggest that this genotype might produce larger families—at least under primitive social and economic conditions.

Partial substantiation of this argument is found in a report by Solth[11] of an appreciable association between the ages of mothers at first delivery and their ages of inception of the menarche. Tabulation of his data follows:

Menarchial ages	Number of cases	Interval between menarche and first delivery	Average age at first delivery
9-12	188	12.1	23.0
13-14	613	10.5	24.4
15-16	316	9.1	25.1
17-22	97	8.4	27.9

Since mothers whose menarchial ages are younger than the average apparently tend to have their first pregnancies earlier, it is not unreasonable to suggest that they may also have larger families, or that they used to in primitive cultures.

COLLECTION OF DATA

An exploratory test of this hypothesis was made by investigating the age of inception of the menarche of two groups of diabetic girls, one group with diabetes onset before age eleven, the other with onset at least two years following the menarche and in no case before eighteen. Both groups are composed of recent patients of the Jos-

TABLE 2

Mean menarchial ages of students from four state Universities by separate year of birth (From Mills, 1941)[11]

In each University series the lowest mean is underlined.

Birth Year	University of North Carolina Number	Mean		P.E.	Kansas University Number	Mean		P.E.
1915	563	13.54	±	.032	318	13.44	±	.048
1916	536	13.52	±	.033	269	13.38	±	.049
1917	641	13.42	±	.032	274	13.32	±	.048
1918	669	13.31	±	.030	291	13.20	±	.051
1919	621	13.34	±	.029	295	13.15	±	.049
1920	600	13.35	±	.030	233	13.23	±	.060
1921	476	13.40	±	.034				
1922	59	13.42	±	.086				
	University of Cincinnati				University of Wisconsin			
1915	301	13.38	±	.044	901	13.47	±	.028
1916	281	13.33	±	.044	991	13.36	±	.028
1917	303	13.31	±	.046	986	13.34	±	.028
1918	339	13.20	±	.045	1,098	13.29	±	.025
1919	246	13.09	±	.049	967	13.28	±	.026
1920	277	13.20	±	.054	896	13.26	±	.028
1921	258	13.13	±	.047	775	13.22	±	.030
1922					280	13.23	±	.050

TABLE 3

Variation of menarchial age with year of birth in two groups of diabetics

Birth year	Diabetes onset at eighteen or over Mean	Median	Number	Diabetes onset before eleven Mean	Median	Number
1915	12.8	12.2	6			
1916	12.9	13.0	8			
1917	12.3	12.1	9			
1918	12.9	12.6	14			
1919	13.1	13.2	9			
1920	13.1	13.1	20	15.4	15.1	9
1921	12.9	13.1	18	13.3	12.9	7
1922	13.1	13.1	9	15.7	15.5	12
1923	13.1	12.8	18	15.0	15.0	13
1924	12.7	12.6	12	14.3	14.3	11
1925	12.5	12.8	14	14.9	15.2	10
1926	13.2	13.1	11	13.6	13.4	11
1927	12.8	12.5	15	14.9	15.0	9
1928	12.8	12.9	5	14.3	14.8	8
1929	13.0	13.2	13	14.0	13.7	6
1930	12.4	12.5	7	14.0	13.6	11
1931	12.1	12.0	8	13.4	13.3	14
1932	13.0	13.8	9	13.3	13.4	6
1933	13.5		2	13.1	13.3	14
1934	11.2	11.5	3	13.3	13.2	12
1935	12.5	12.3	3	12.9	13.1	12
1936	13.6		1	14.1	14.2	21
1937	13.3		1	13.7	13.3	14
1938	13.5		2	13.4	13.1	18
1939	12.7		1	12.8	12.0	7

lin Clinic in Boston. Patients born or residing over 300 miles from Boston, nonwhite patients and patients having other serious illness prior to age twenty were excluded to attain greater homogeneity. Birth dates ranged from 1915 to 1939. All diagnoses were confirmed by Dr. Elliott P. Joslin personally.

Data were derived from returns of a mailed questionnaire inquiring (1) "What was the date of your first menstrual period? ——— (Year) ——— (Month)." (2) "How old were you then? ——— (Years) ——— (Months)." (3) "Is your answer to the above correct to within one month, or two months, or three months? ——— Or, more than three months? ———."

About one quarter of the addressees failed to respond to the questionnaire, even upon second request. Replies were eliminated if not consistent with the date of birth

on file at the Clinic, or if not stated to be correct to within three months. These constituted a second quarter. The remaining half are believed to be satisfactorily reliable, since almost all of the Clinic's patients have proved themselves to be conscientious, and almost all of those who answered the questionnaire had been corresponding with the Clinic in answer to periodic letters concerning the course of the diabetes, response to changes in therapy, complications, pregnancy and other matters. Many replies mentioned incidents or occasions which had fixed the date of menarche in mind. Some were based on the memories of the mother or older relatives or friends, some on diaries.

Controls were obtained from records of medical examinations of Wellesley College students born and residing within 300 miles of Boston, excluding those of non-European descent. Results were subdivided by year of birth, since this factor has been shown to be closely associated with menarchial age.[12]

Several weaknesses of the data should be indicated. (1) While the failure of about one quarter to answer the questionnaire may be attributed to indifference, indisposition, ignorance etc., there is no evidence that those who did reply were an unbiased sample of the total. With a second quarter eliminated for inaccuracy the possibility of bias in the remaining half cannot be overlooked. (2) The ages at which patients made their replies varied from eighteen to forty, while the ages of the Wellesley College students at the time of examination varied from eighteen to twenty. This differential in age may introduce a bias. (3) The students were asked about their menarchial age by personal contact, and were not permitted to avoid making reply.

RESULTS AND COMPARISONS

The two groups of diabetics are clearly differentiated in average menarchial age, the group with onset of diabetes after adolescence coming into the menarche 1.1 years earlier than those with childhood onset, a difference which is about seven times its standard error and may be considered significant, viz:

	Number	Mean	S.E. of Mean
Diabetes before eleven	222	13.96	±0.127
Diabetes at eighteen or over	219	12.84	±0.086
Difference		1.12	±0.053

The diabetics with childhood onset have an average menarchial age which is higher than that of any of the most comparable groups found in the literature. The highest mean of any such group is 13.54 years ± 0.032 (P.E. of mean) reported by Mills[12] for 563 students at the University of North Carolina born in 1915. The difference of 0.42 years is over three times its standard error and may be considered significant (t=3.8, P under .001).

The diabetics with postadolescent onset have an average menarchial age which is lower than the Wellesley student controls' average by 0.33 year, a difference which is over three times its standard error and may also be considered significant:

	Number	Mean	S.E. of Mean
Wellesley College students	1,185	13.17	±0.033
Diabetes at 18 or over	219	12.84	±0.086
t=3.8 P under .001		0.33	±0.092

Only eighty-three diabetics with postadolescent onset were born during the seven birth years of the Wellesley students, 1916-1922. These eighty-three have an average menarchial age of 12.89 ± 0.15 years which is not different from that of the remainder of the sample.

The most comparable groups found in the literature are students of four state universities subdivided by year of birth reported by Mills.[12] Their average menarchial ages for the years of birth 1915-1922 are presented in table 2. Not one birth year group has an average menarchial age as low as that of the diabetics with postadolescent onsets, nor as high as that of the childhood onset diabetics.

These differences are presented graphically in figures 1 and 2.

The variation of menarchial age with year of birth of both groups of diabetics is shown in table 3. Since the number of patients in each birth year is small, the medians are given as well as the means.

Two conclusions may be drawn from the above: (1) that childhood diabetes tends to retard the inception of the menarche, and (2) that girls having the *dd* genotype come into the menarche at somewhat earlier ages than the students, provided they have no diabetes until at least two years following the menarche inception. This situation suggests that possibly the *dd* genotype produces a pleiotropic effect. (Incidentally, since approximately 5 per cent of students would be expected to have the *dd* genotype, insofar as the entire population is estimated to have this frequency, and students might be expected not to include very many juvenile and adolescent cases, therefore the difference of 0.33 years shown above may be regarded theoretically as a maximum.) The statistical significance of the second conclusion is uncertain, however, because of the possibilities of bias described above.

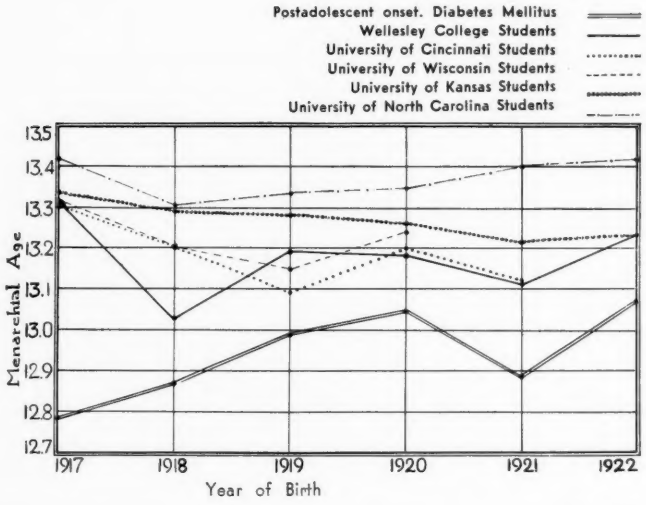

FIG. 1. Menarchial ages of girls diabetic at eighteen or over, compared with five Student Groups, subdivided by Year of Birth.

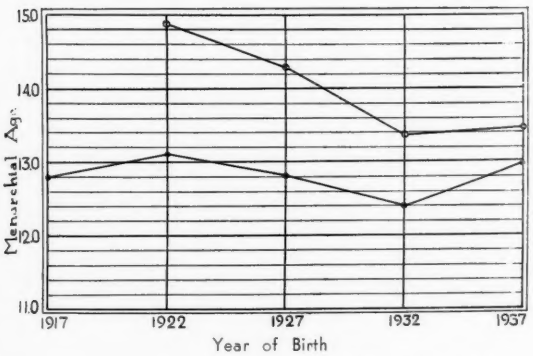

FIG. 2. Menarchial ages of diabetics with onsets at age eighteen or over (●) and before age eleven (o).

SUMMARY

Average menarchial age of 222 girls who developed diabetes before age eleven is 13.96 years with standard deviation 1.89 years. This is later than the average of any comparable group known. It suggests that childhood diabetes may retard the inception of the menarche.

Average menarchial age of 219 girls who developed diabetes at eighteen or over is 12.84 years, with standard deviation 1.27 years. This group, with diabetes onset following adolescence, has an average menarchial age earlier than that of a control of Wellesley College students, and earlier than that of any comparable group of non-diabetics found in the literature. This early maturity may be attributable to a pleiotropic effect of the diabetes-producing genotype which is apparently unconnected with the overt disease. However, comparison with student groups is not fully justifiable because of possibilities of bias in the collection of data.

SUMMARIO IN INTERLINGUA

Explication Tentative del Alte Incidentia de Diabete Mellite

Le etate medie al tempore del menarche in 222 pueras qui disveloppava diabete ante le etate de dece-un annos esseva 13,96 annos, con un deviation standard de 1,89 annos. Isto excede le correspondente etate medie in omne comparabile gruppo unquam reportate. Se impone le notion que diabete juvenil retarda le inception del menarche.

Le etate medie al tempore del menarche in 219 pueras qui disveloppava diabete a o post le etate de dece-octo annos esseva 12,84 annos, con un deviation standard de 1,27 annos. Iste gruppo, in que diabete se declarava post le adolescentia, habeva un etate medie al tempore del menarche infra le correspondente etate medie de un gruppo de controlo consistente de studentes del Collegio Wellesley e infra illo de omne altere gruppo de non-diabeticas trovate in le litteratura. Iste precoce maturitate es possibilemente attribuibile al effecto pleiotropic del genotypo diabetogene, un effecto que apparentemente es sin connexion con le morbo in su manifestationes patente. Tamen, le comparation de gruppos de studentes non es integremente justificabile a causa del possibilitate de preconditionamento distortive per le selection del datos.

ACKNOWLEDGMENTS

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Race, Sex and Fatal Acute Myocardial Infarction

The over-all incidence of acute myocardial infarction was five times as high among the whites as among Negroes. The difference was much greater in the period 1940 to 1954 than in the period 1910 to 1939 because the incidence among white individuals had risen tremendously but that among Negroes had risen only slightly. The difference in incidence increased with increasing age because the incidence rose sharply among whites with increasing age but showed little change among Negroes. The increasing difference in the incidence of acute myocardial infarction with increasing age is not simply a matter of difference in longevity, because the data on incidence refer to the percentage incidence among those who survive to a given age. For example, more than 25 per cent of a group of white women who reached the age of seventy died of myocardial infarction and the corresponding figure for a group of Negro women over seventy years old was only 2 per cent. The incidence of acute myocardial infarction among Negroes was similar in the two sexes in both periods that were studied. . . .

No evidence was found to indicate that the changing sex ratio of acute myocardial infarction could be accounted for by a changing incidence of diabetes mellitus, hypertension, or obesity. However, it should be pointed out that in both periods (1910 to 1939 and 1940 to 1954) the relative incidence of acute myocardial infarction in the two sexes was different among diabetic patients than among nondiabetic patients (among diabetic patients before 1940 the sex ratio for acute myocardial infarction was 1:1, but after 1940 there was a preponderance of women; among nondiabetic patients before 1940 the sex ratio was 2.3 women to 1 man and after 1940 it was 1.4:1). Although a change has occurred in the relative sex incidence among diabetic patients and nondiabetic patients, acute myocardial infarction is still slightly more common among male nondiabetic patients than among female nondiabetic patients and it is slightly more common among female diabetic patients than among male diabetic patients.

From "Fatal Acute Myocardial Infarction: Sex, Race, Diabetes and Other Factors," by Wilbur A. Thomas, M.D., in *Nutrition Reviews*: *15*:4, April 1957, pp. 97-101.

The Reproductive Characteristics of Diabetic Men

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It is now well recognized that a woman suffering from diabetes mellitus may transmit a genetic background for the disease to her offspring.[1] It is also appreciated that the disease may have an overt unfavorable influence upon a diabetic woman's reproductive capacity, as exemplified by an increased incidence of abortions, stillbirths, intrapartum and neonatal deaths, and excessive-sized fetuses.[2] An abnormally high incidence of female offspring is also reputed to occur. It has been assumed that all of these reproductive abnormalities stem from the mother's unfavorable internal environment. However, the possibility remains that genetic factors may play a role in at least some of these occurrences. It is also possible that diabetes in the father may by a genetic influence contribute to such misfortunes of reproduction. In support of this, Jackson[3] has recently reported from South Africa that infants of diabetic fathers are significantly heavier at birth than infants of nondiabetic men. Unfortunately, little else has been written concerning the influence of diabetes mellitus on the reproductive capacity of men. The scattered reports deal with the 17-ketosteroid excretion of diabetic men,[4] the occasional testicular atrophy[5] and calcification of the vas deferens,[6] and the clinical impression of an increased incidence of impotence.

In the present investigation, 167 diabetic men were studied in relation to their reproductive histories. Data pertaining to the high incidence of impotence discovered among these men are given elsewhere.[7] Material concerning their fertility, and the outcome of all the conceptions of their wives are presented here.

MATERIALS AND METHODS

One hundred sixty-seven married men with diabetes mellitus served as subjects. The men were chosen at random from the Diabetes Clinics at the Hospital of the University of Pennsylvania and at the Philadelphia General Hospital. Their ages ranged from sixteen to ninety-two years. Their ages at the onset of diabetes ranged from two and one-half to seventy-eight years. Each subject was interviewed with respect to his reproductive history. His age at marriage, use of contraception, the number of pregnancies engendered, their clinical course and outcome, the age and health of his spouse, and so on, were ascertained. Information concerning the subject's diabetic status and general health were obtained from his medical record. If there was a history of diabetes in his wife, the subject was excluded from the study. Data for controls were obtained by applying the same interview technic to 157 married men attending the Outpatient Clinics of the University of Pennsylvania School of Dentistry. Individuals who were known to have diabetes or whose wives had diabetes were excluded.

Fertility (Table 1). The wives of the 167 diabetic men had 553 conceptions, compared with 356 conceptions for the wives of the 157 nondiabetic men. The diabetic group had an average of 3.2 conceptions for each couple compared with 2.2 for the control population. However, in each group there were individuals who had married too late in life to reproduce, or who were young enough so that they might not have completed their reproductive careers. Therefore, in order to compare better the number of conceptions and other fertility data for the diabetic and control populations, the actual number of years of exposure to the possibility of conception was computed for each group. This was done by calculating for each couple the number of years of cohabitation without contraception prior to the age of forty-five for the husband and forty for the wife. The resulting figure was the number of years of exposure for that couple. The average number of conceptions per year of exposure was found to be 0.221 for the diabetic men and 0.216 for the control series. This difference is not statistically significant (t test). However, the diabetic population included many individuals who were not known to have had diabetes during their reproductive years. In an attempt to determine whether or not there was a difference between the "pre-

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*For this work Dr. Babbott received the Nathaniel N. Wolfsohn Award of the Philadelphia Diabetic Society.

TABLE 1

Comparison of fertility of wives of diabetic and nondiabetic men

Characteristics	Wives of Diabetic Men Number	Per cent	Wives of Nondiabetic Men Number	Per cent
Population	167		157	
Total conceptions	553		356	
Conceptions per family	3.2		2.2	
Conceptions per year of exposure*	0.221		0.216	
Abortions	90	16.6†	39	11.0
Premature births	22	4.8	18	5.7
Stillbirths	12	2.9	8	2.2
Twins	5		4	
Term pregnancies	441		299	
Male‡	237	54	143	48
Female	200	46	153	52
Barren marriages§	28	18.3	24	16.1

*Years of cohabitation without contraception prior to the age of forty-five years for the husband and forty years for the wife.

†Significantly different from nondiabetic group (x square, P = <.01).

‡Stillbirths are excluded from the totals by sex, since the sex was not always known.

§The couple must have been cohabiting without contraception for at least two years by the age of forty-five years for the husband and forty years for the wife without a conception.

diabetic" and diabetic state in regard to fertility, the twenty-two men known to be diabetic before the age of forty-five were studied. This group averaged 0.250 conceptions per year of exposure, a figure comparable to the two others cited above.

There was no statistical difference in the incidence of barren marriage among the diabetic men compared with the control population. (Barren marriage was defined as at least two years of cohabitation without contraception before the age of forty-five for the husband and forty for the wife without a conception.) Eighteen and three-tenths per cent of the diabetic men had barren marriages, compared with 16.1 per cent in the control population.

Abortion (termination of pregnancy prior to the 28th week of gestation). The wives of the diabetic men had 553 conceptions. Sixteen and six-tenths per cent of these ended in abortions. The wives of the control population had an abortion rate of 11 per cent in 356 conceptions. Thus, the wives of the diabetic men had a significantly higher incidence of abortions (chi square =7.1, P=<.01). The rate of abortion in the wives of the diabetic men after the clinical recognition of the diabetes in their husbands did not differ significantly from the incidence prior to the discovery of the husband's disease. To see if a possible difference in the ages of the diabetic and control populations might be a factor in the higher abortion rate of the diabetic group, the average ages of the husband and wife at marriage were compared for the diabetic and control groups. These did not differ significantly.

Premature Births, Stillbirths and Sex Ratio of Offspring. The incidence of premature births, stillbirths and the sex ratio of the offspring of the diabetic men did not differ significantly from that of the control population, or from the usually anticipated incidence.

Birth Weights of Offspring (Table 2). No significant differences were found between the average birth weights of the children of diabetic fathers and those of the control fathers (chi square analysis). The weights of the offspring of the diabetic men also compare well with the most recent data on birth weights for the United States as

TABLE 2

Comparison of birth weights of offspring of diabetic and nondiabetic men

Population	Number of children	Distribution of Children by Weight (cf. Jackson[3]) Less than 8 lbs.	8-8.9 lbs.	9-10 lbs.	Over 10 lbs.
		Per cent	Per cent	Per cent	Per cent
Diabetic	265	72.5	14.3	7.5	5.7
Nondiabetic	245	71.4	17.6	6.9	4.1
United States, 1951*	3,000,000	66	26	7	2
United States Weight Criteria		(Less than 7 lb. 12 oz.)	(7 lb. 12 oz.-8 lb. 13 oz.)	(8 lb. 14 oz.-9 lb. 14 oz.)	(9 lb. 15 oz. or more)

*United States Department of Health, Education, and Welfare. Birth Weight Statistics, United States, 1951. Government Printing Office, Washington, 1954.

published by the Office of Vital Statistics.[8] The latter report, it should be pointed out, is compiled from birth certificates, and uses a slightly different weight classification.

DISCUSSION

The only abnormality of the reproductive process discovered in the wives of the diabetic men was a significantly higher abortion rate than that of the control population. Although the accuracy of the information supplied by husbands concerning their wives' abortions may be questioned, there is no reason to believe that the data supplied by the diabetic men differed in reliability from that given by the nondiabetic men.

There were no differences in the birth weights of the offspring of the diabetic and control populations. Thus, the present study does not support recent work which indicated that children of diabetic fathers were larger at birth than those of a control series.[3] However, the inherent inaccuracies of determining birth weights by an interview technic such as was used in the present study, as well as in the previous work just alluded to, should be recognized.

SUMMARY

One hundred and sixty-seven married men suffering from diabetes mellitus were chosen at random from two outpatient diabetes clinics. They were interviewed with respect to their reproductive histories. The data were compared with those obtained by similarly interviewing a control population selected at random from among married nondiabetic men attending an outpatient dental clinic. The diabetic men's wives had a significantly higher incidence of abortions than did those of the control population. The incidence of abortion among the wives of the diabetic men before and after the clinical recognition of the husbands' diabetes did not differ significantly. The diabetic group did not differ significantly from the control population with respect to number of conceptions, premature births, stillbirths, live births, sex ratio of offspring or birth weights of offspring. Thus, no evidence was found to support the thesis that the children of diabetic men differ discernibly at birth from those of normal men.

SUMMARIO IN INTERLINGUA

Le Characteristicas Progenitori De Masculos Diabetic

Cento sexanta-septe masculos maritate qui suffreva de diabete mellite esseva seligite al hasardo ab inter le patientes visitante de duo clinicas pro diabete. Illes esseva questionate con respecto a lor historias progenitori. Le datos assi colligite esseva comparate con un collection correspondente obtenite per similemente questionar un population de controlo seligite al hasardo ab inter le visitante nondiabetic patientes mascule maritate de un clinica dental. Le feminas del diabeticos habeva un significativemente plus alte incidentia de abortos que le population de controlo. Le indicentia del abortos inter le feminas del diabeticos ante e post le recognition clinic de lor stato diabetic non differeva significativemente. Le gruppo diabetic non differeva significativemente ab le population de controlo. Le gruppo diabetic non differeva significativamente ab le population de controlo con respecto al numero de conceptiones, de parturitiones prematur, de morte-natos, de nascentias normal, del proportion del sexos in le prole, e del pesos natal. Assi nulle prova esseva trovate in supporto del these que le prole de masculos diabetic differe appreciabilemente ab le prole de masculos normal al tempore del nascentia.

ACKNOWLEDGMENT

It is a pleasure to acknowledge the cooperation of Doctors Lester W. Burket, W. Wallace Dyer, Francis D. W. Lukens, and Anthony Sindoni, Jr., in making clinical facilities available for this study.

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Paperchromatography, a Simple Method for the Differentiation of Sugars in the Urine

A Preliminary Report

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Paperchromatography has proved to be a simple, cheap and reliable method of separating the various sugars which occur alone or in combination in urine. During the last few years, I have used it following Isler's modification of the method described by Riffart, Decker and Wagner.[1, 2] It is based on the fact that various sugars migrate with different speeds, and that the R_f is characteristic for each individual sugar under the same conditions.

On a rectangular filter paper (Whatmann No. 1 about fifteen inches long) a baseline is drawn by pencil approximately one and one-half to two inches from the lower margin. With a small pipette sufficient urine is applied to moisten an area of about one-quarter inch in diameter and let dry. If the urine has given a green Benedict's test, this is repeated on the same spot for about five times, always awaiting dryness of the preceding load. Two applications are sufficient when the Benedict's test was yellowish-green and only one with urine containing still more sugar. More laterally about one inch apart, one deposits on the baseline one drop of various test sugars: pentoses such as xylulose (xyloketose), arabinose, xylose and ribose; hexoses such as glucose, fructose and galactose; and disaccharides such as maltose, lactose and sucrose. The test solutions are prepared by dissolving 0.1 gm. of the sugar in 2 ml. of a mixture of water with concentrated alcohol in equal parts. They are kept in the refrigerator and renewed every two months. In order to compensate for the influence of temperature, of salts, protein and other substances which modify migration in the filter paper, one always runs parallel to the urine a sample of urine to which the sugar or sugars in question have been added.

So far we have not used the method for quantitative measurements; for qualitative examination of urine with positive Benedict's test this method is sufficient. Quantitative analyses demand accuracy in measuring the amount of urine analyzed and of the test sugars applied, perhaps also desalting.

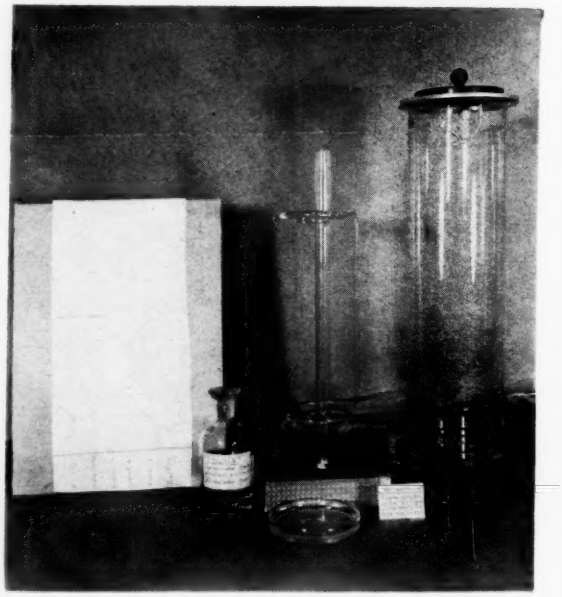

FIG. 1. Equipment for the differentiation of sugars in the urine by paperchromatography.

When the test drops have dried, the filter paper is fixed on a coiled frame (figure 1). Contrary to the original method, this device designed by Isler[2] permits the use of a relatively large filter paper, up to eighteen inches, and the simultaneous analyses of several urine specimens. At least twelve hours previously the bottom of a glass cylinder (figure 2) is filled with a solvent containing seven parts of isopropyl alcohol, two parts glacial acetic acid and one part water in order to saturate the air with vapor. On an H-shaped grate rests a Petri dish into which the coiled frame with the mounted filter paper is placed. The lower border of the filter paper has to touch the floor of the Petri dish evenly, but it should not contact the brim. Through the central glass tube to which the coiled frame is fixed, solvent is poured into the Petri dish, about one-half inch deep. Now the glass cylinder is closed and kept in a room and

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TABLE 1
R_f values of various sugars

Lactose	0.1	Mannose	0.37
Maltose	0.13	Fructose	0.38
Sucrose	0.20	Arabinose	0.39
Galactose	0.26	Xylose	0.42
Glucose	0.30	Ribose	0.49
	Rhamnose	0.57	

TABLE 2
Findings in 250 urines from 186 individuals

	Diabetics	Nondiabetics
Glucose alone	148	12
Arabinose alone	6	5
Xylose alone	8	8
Xylulose alone	4	1
Fructose alone	1	
Lactose alone (pregnancy)	1	
Glucose + arabinose	25	3
Glucose + xylose	14	3
Glucose + xylulose	3	
Glucose + fructose	2	
Glucose + lactose	2	
Galactose + arabinose		2
Lactose + arabinose	1	
Glucose + arabinose + lactose	1	
	216	34

a place where on all sides the surrounding temperature is the same, to insure an even migration of the solvent. Before the front of the latter has reached the top—at ordinary room temperature this will be in about twenty-four hours—the paper is removed from the cylinder and air-dried, then sprayed with a solution containing 0.5 per cent benzidine, 20 per cent glacial acetic acid and 79.5 per cent absolute alcohol. After this, the paper is laid for fifteen minutes in an oven or sterilizer at 110° C. Pentoses show a reddish color, whereas fructose, glucose, maltose and lactose appear brown.

The purpose of this preliminary report is to draw attention to a simple method of separating reducing substances in urine and thus differentiating glucosurias from other meliturias. The simultaneous occurrence of other sugars besides glucose in the urine of diabetic patients has, to my knowledge, not been sufficiently investigated to permit definite conclusions. It appears, however, that alimentary pentosuria (arabinose, xylose) is not very rare in diabetics in contrast to essential pentosuria (xylulose) which is seldom met in combination with diabetes mellitus.

SUMMARIO IN INTERLINGUA

Chromatographia A Papiro—Un Simple Methodo Pro Le Differentiation De Sucros In Le Urina

Le autor describe un simple, incostose, e fidel methodo de chromatographia a papiro pro separar le varie sucros que occurre individual- o combinatemente in le urina. Le methodo es identificate como le modification que Isler effectuava in le methodo originalmente describite per Riffart, Wagner, e Decker. Usque al presente, le methodo ha essite usate exclusivemente pro examinationes qualitative. Le objectivo del autor in presentar su reporto es signalar le importantia e le practicabilitate de differentiar le glucosurias ab altere formas de meliturias.

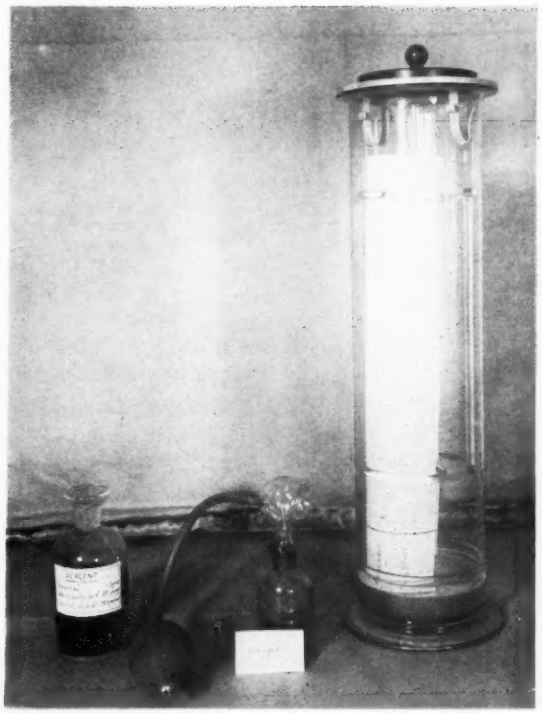


FIG. 2. Filter paper fixed to the frame and placed into the glass cylinder.

REFERENCES

[1] Riffart, W., Decker, T., and Wagner, H.: Klin. Wchnschr. 30:350, 1952.

[2] Constam, G. R., and Isler, K. M.: Gastroenterologia. 87: 145-49, 1957.

[3] Peters and Van Slyke: Quantitative Clinical Chemistry, Interpretations, Vol. I, London 1946.

DISCUSSION

DAVID W. KRAMER, M.D., (*Philadelphia*): Were you able to pick up any of the other forms which might be in the carbohydrate group, but particularly the polysaccharide or mucopolysaccharide with this method?

DR. CONSTAM: We didn't try any mucopolysaccharide, but we get saccharose and maltose.

We have tried the same method to differentiate the sugars in the blood. We tried first by electrophoresis to split up the proteins in the blood, and then separated the sugars by "second dimension chromatography."

A Comparison of the Behavior of Insulin and Insulin Labeled with I^{131} in Serum

G. W. Scott, M.D., T. E. Prout, M.D., J. A. Weaver, M.D., S. P. Asper, M.D.
Baltimore

The use of insulin labeled with iodine (I^{131}) has facilitated study of the factors influencing insulin metabolism.[1, 2, 3] The rate of disappearance of intravenously administered insulin I^{131} has been studied by two methods. In the first of these, the radioactivity of the precipitate obtained by the addition of trichloroacetic acid to the plasma has been measured. This is the generally accepted method of study. In the second method, recently introduced by Berson and his associates,[3] electrophoresis of the serum has been employed to separate insulin I^{131} from the plasma for measurement. By this technic, they identified a major fraction as unaltered insulin I^{131} and, in addition, a minor fraction of insulin I^{131} which had a different physical behavior. Employing the two methods, these workers studied rates of disappearance of insulin I^{131} given by intravenous injection to rabbits. The rate of disappearance of the radioactivity of the TCA precipitate was much slower than the rate of disappearance of the major radioactive fraction of insulin I^{131} as measured by electrophoresis. The persistence in the circulation of the minor fraction of insulin I^{131} appeared to account for the difference in behavior of insulin I^{131} when studied by these two technics.

Since changes in the structure of insulin might affect its biologic activity, it appeared worthwhile to determine the rate of disappearance of administered unlabeled insulin by means of a bio-assay. The studies of the disappearance of insulin I^{131} from the blood as calculated from measurements of radioactivity have been repeated and compared with a study of the disappearance of unlabeled insulin as determined by biological assay.

METHODS

Insulin I^{131}. The radioactive insulin used was obtained from a commercial source.* Prior to iodination the insulin† was stated to have a biologic activity of 27 units per mg. The specific radioactivity at the time of use varied between 2.4 and 8.2 microcuries of I^{131} per microgram of insulin. Both dialysed insulin and nondialysed insulin were used. Dialysis was carried out against running water for periods of twelve to forty hours; this increased the TCA precipitable radioactivity of the original insulin sample from 88 per cent to 97 per cent. The insulin molecule was stated to have one atom of iodine per mole assuming its molecular weight to be 6,000.

Trichloroacetic Acid Precipitation: Insulin I^{131} was injected intravenously into rabbits in doses from 0.08 to 0.15 units of insulin per kg. body weight. This gave an approximate radioactivity of 20 microcuries per kg. body weight. Blood samples were taken from the central artery of the ear at ten-minute intervals from ten to seventy minutes following injection. In some experiments, samples were taken for periods up to 250 minutes. The precipitable radioactivity was obtained by adding one ml. of 20 per cent trichloroacetic acid to 0.05 ml. of plasma. After centrifugation the precipitate was washed twice with 20 per cent trichloroacetic acid, recentrifuged and the supernatant poured off. The precipitate was dissolved in 30 per cent KOH with heat. This solution was poured into a metal planchet; in order to insure transference of all the radioactivity the centrifuge tube was washed several times with water containing a detergent and the washings were added to the planchet. The solution was evaporated to dryness, following which the radioactivity was measured by means of a shielded Geiger-Muller tube and scaling unit.

Paper electrophoresis: Paper electrophoresis with Whatman No. 3 MM filter paper was carried out in 0.05 molar barbital buffer (*p*H 8.6) at a constant voltage of 115 volts. Runs lasted for eight to ten hours.

Presented in part at the Seventeenth Annual Meeting of the American Diabetes Association in New York City on June 1, 1957.

From the Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland.

*Abbott Laboratories.

†Eli Lilly crystalline zinc insulin.

When the radioactivity at the point of application, or origin, was studied, however, no current was applied but the plasma proteins were separated from the insulin on the filter paper strips by hydrodynamic flow as previously described.[3] After electrophoresis the filter paper strips were heated for thirty minutes at 100° C. and stained with bromphenol blue followed by washing with 5 per cent acetic acid. The various protein fractions were identified and cut separately from the strips. The radioactivity of these and of the origin was then determined.

Biologic Assay: A biologic assay of insulin was carried out, using the rat hemidiaphragm technic modified from Stadie and Zapp.[4] The rats used in the assay procedure were of the Sprague-Dawley strain. The animals were fasted for a period of sixteen to twenty hours preceding the experiment. Only male rats were used, their weights varying from 150 to 200 gm. Each rat was killed by stunning and decapitation, and the diaphragm was quickly removed and placed in a salt solution buffered with bicarbonate and containing 250 mg. per cent glucose.[5] The thick crura and central tendon were excised and the diaphragm was bisected. The hemidiaphragms were trimmed to similar weights. After removal of excess fluid by laying on filter paper, the hemidiaphragms were weighed on a Roller-Smith balance. The average weight of the hemidiaphragms was 172 mg. with an average difference between pairs of 2.6 mg. Glucagon-free insulin* was freshly diluted in the glucose-salt solution immediately prior to use in concentrations from 2.5 to 80 milliunits per ml. One hemidiaphragm was placed in 0.4 ml. of glucose-salt solution containing insulin and shaken gently for two minutes at 37° C. The hemidiaphragm was then washed in glucose-salt solution and blotted lightly with filter paper. The other hemidiaphragm was similarly treated with the exception of the exposure to insulin. Both hemidiaphragms were placed in separate bottles containing 1 ml. of the glucose-salt solution. Since it is difficult to maintain the bicarbonate buffer at a physiological *p*H, the solution was gassed prior to use with 90 per cent oxygen and 10 per cent carbon dioxide. In addition, the bottles were gassed again for several minutes after receiving the hemidiaphragms and were then tightly stoppered. They were incubated at 37° C. for one hour with gentle rocking.

Analyses of the glucose concentration of the incubating fluid were determined in duplicate by a modified glucose-oxidase method[6, 7] before and after incubation. The glucose uptake of each hemidiaphragm was expressed in mg. of glucose per gm. of diaphragm per hour. The increase in glucose uptake of the hemidiaphragm exposed to insulin above that of the control was plotted against the concentration of the insulin.

Assay of the insulin content in the sera of the rabbits was carried out before and after the intravenous injection of crystalline zinc insulin. Doses ranging from three to fifty units per kg. body weight were used, and sera were collected at intervals up to 140 minutes following injection. The insulin content of the sera was determined by the rat hemidiaphragm technic as described above. One hemidiaphragm was placed in 0.4 ml. of serum in a small test tube and incubated at 37° C. for two minutes with gentle shaking. It was then washed in 5 ml. of glucose-salt solution for five minutes and lightly blotted on filter paper. The hemidiaphragm was then placed in glucose-salt solution as previously described. The control hemidiaphragm was similarly handled with the exception of the exposure to serum. The results were expressed as the increase of glucose uptake of the hemidiaphragm treated with serum above that of the control in mg. of glucose per gm. of diaphragm per hour. For each of the sera duplicate or occasionally triplicate diaphragm assays were made.

The blood sugars of the rabbits during the experiments were determined by the anthrone method.[8]

RESULTS

With electrophoresis or hydrodynamic flow, the main fraction of insulin I^{131} remains adsorbed to the filter paper at the point of application. The adsorption to the paper is unaffected by the presence of plasma proteins in normal concentrations. Following intravenous administration of insulin I^{131}, the disappearance of the radioactivity from the serum is rapid for the first twenty-five to thirty-five minutes, presumably due to distribution of insulin in the available body space. Figure 1 shows the electrophoretic pattern of the serum of a rabbit thirty-one minutes after intravenous injection of insulin I^{131}. The strip has been scanned for its protein fractions, and the amount of radioactivity with the various fractions has been measured. The major radioactive fraction remains adsorbed at the origin, and variable amounts of radioactivity migrate with the plasma proteins. Since the strip has been stained and washed, only protein-bound radioactivity remains, and a part equivalent to 28 per cent of the precipitable radioactivity migrates with the proteins. The major radioactive fraction adsorbed at the origin disappears uniformly between thirty and seventy minutes whereas the radioactive frac-

*Kindly supplied by Eli Lilly and Company, Indianapolis, Indiana.

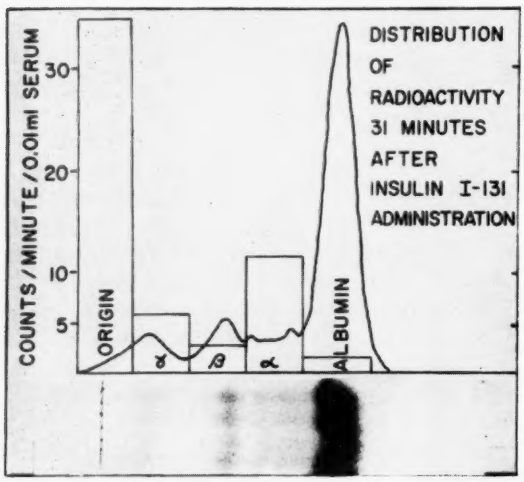


FIG. 1. Paper electrophoresis of serum after intravenous administration of insulin I^{131}. Vertical columns represent radioactivity along the strip. The point of application is designated "origin."

tion migrating with the proteins shows little change over this period of time.

Since the TCA precipitate includes the material at origin and the radioactivity migrating with the proteins, the persistence of the latter fraction in the serum leads to a slower rate of disappearance for the TCA precipitate than for the fraction at origin alone (figure 2).

The mean half life of insulin I^{131} calculated for the period of thirty to seventy minutes following injection is sixty-seven minutes by the technic of TCA precipitation and twenty-four minutes by electrophoresis. At later periods of time the TCA precipitable radioactivity disappears at progressively slower rates until it corresponds to that of the fraction migrating with the plasma proteins. Over short periods of time the migrating fraction shows no detectable degradation, and the difference in absolute counts per minute between the fraction remaining at origin and the TCA precipitate is constant. In most experiments this constant difference was present throughout the period of study (figure 3). In some experiments a constant difference could only be demonstrated in the later determinations, i.e., after forty minutes, suggesting that in these cases contact with the serum had some effect in producing the migrating component (figure 4).

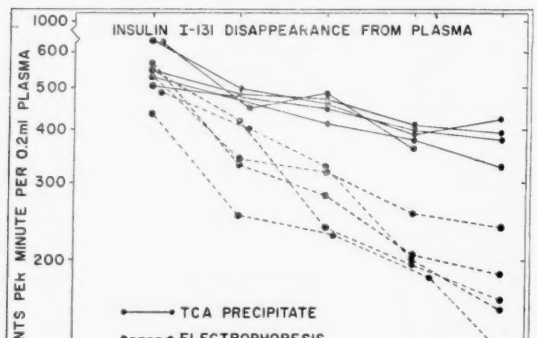
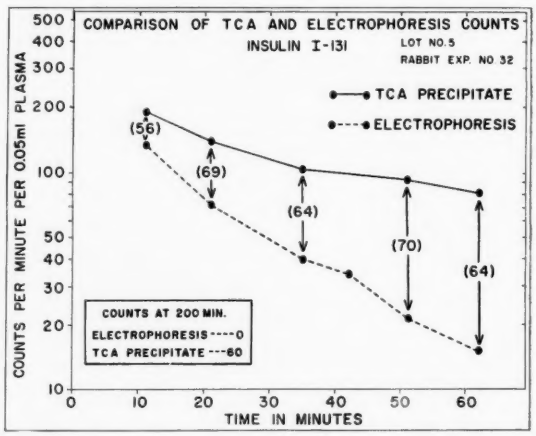
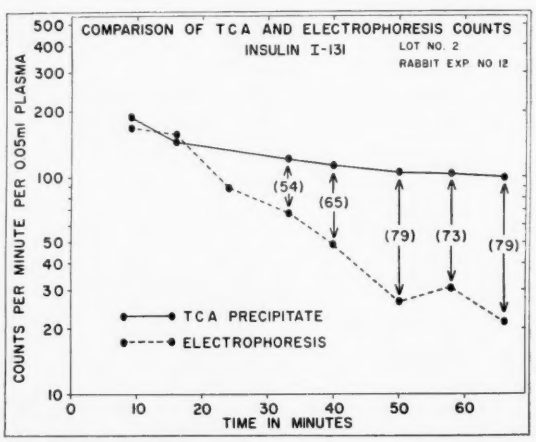


FIG. 2. Comparison of the rates of disappearance of insulin I^{131} from plasma in vivo as measured by trichloroacetic acid precipitation and paper electrophoresis.





FIGS. 3 & 4. Differences are shown by figures in parentheses, in absolute counts per minute, between the trichloroacetic acid precipitate and paper electrophoresis. These differences are equivalent to the radioactivity migrating with the protein fraction.

The disappearance rate of insulin I^{131} is not changed by the simultaneous administration of large doses of insulin; crystalline zinc insulin in doses of 100 units per kg. body weight had no effect on the rate of disappearance of radioactivity from the plasma (figure 5). Berson, Yalow and Volk[9] have demonstrated that the degradation mechanisms are saturated only with doses exceeding 200 units of crystalline zinc insulin per kg. body weight.

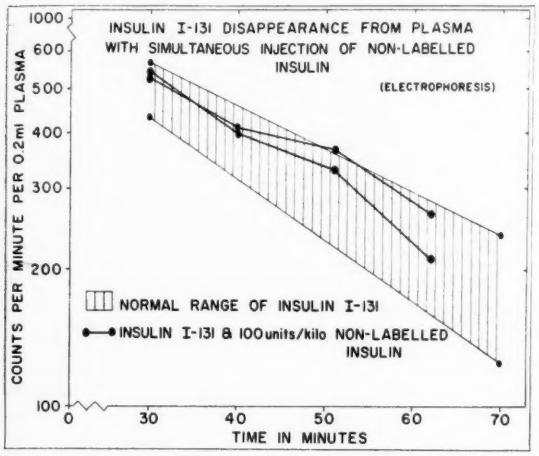


FIG. 5. Comparison of the rate of disappearance of insulin I^{131} alone and with simultaneous injection of large doses of unlabeled insulin.

A theoretical space of distribution for insulin I^{131} has been calculated by extrapolation to zero time of the exponential decay curve for electrophoresis. This is based on the assumption that the disappearance rate is constant from the time of injection. The average space of distribution was 25.8 per cent of body weight. This calculation of a theoretical space of distribution does not imply that the insulin is uniformly distributed throughout this space.

It appeared likely that biologically active insulin I^{131} in serum might be quantitated by its ability to bind itself to the rat hemidiaphragm. A modification of the technic described by Stadie, Haugaard and Vaughan[10] was used. Rat quarter-diaphragms were dipped for two minutes in 0.4 ml. of sera taken at thirty, forty, fifty and sixty minutes from three of the rabbits which had received insulin I^{131}. The diaphragms were then washed for five to six hours in large volumes of normal saline. The radioactivity was determined after digestion of the diaphragms with 30 per cent hot KOH. The radioactivity adsorbed on the muscle was relatively constant up to 250 minutes after injection, in contrast to the decrease in radioactivity of the electrophoretic fraction at origin and TCA precipitate (figure 6). Insulin used in this experiment was dialysed prior to injection. The finding that the radioactivity bound to the diaphragm exceeded that at origin at 250 minutes indicates that excess radioactivity came from either the total TCA precipitable or free radio-iodine liberated during degradation.

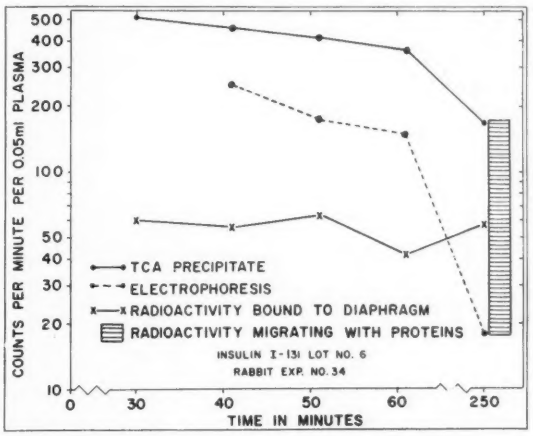


FIG. 6. Comparison of the concentration of radioactive insulin in plasma as measured by trichloroacetic acid precipitation, paper electrophoresis and binding to rat diaphragm.

Insulin I^{131} was mixed with 1/40 molar cysteine in vitro for twenty-four hours in order to inactivate the insulin.[11] It was found that this insulin was bound to the rat diaphragm to the same degree as untreated insulin I^{131}.

In order to assay for biologically active insulin in serum, a log dose response curve of the rat hemidiaphragm to various concentrations of insulin was constructed as outlined in the methods (figure 7). It should be noted that in this procedure, the diaphragms were incubated in the concentrations of insulin for two minutes at 37° C. prior to incubation in the buffered salt solution. There is a curvilinear relationship between the log concentration of insulin and the increase in uptake of glucose in mg. per gram of diaphragm per hour. The slope of the linear portion of the response between five and forty milliunits per ml. is 1.44 with a combined standard deviation about means of 0.54, giving an index of precision of 0.37. At concentrations lower than 2.5 milliunits per ml. it was found that the increase in glucose uptake approached the limits of accuracy for glucose estimation.

In preliminary experiments in which insulin was administered intravenously to rabbits in doses of three units per kg. body weight, it was found that in some

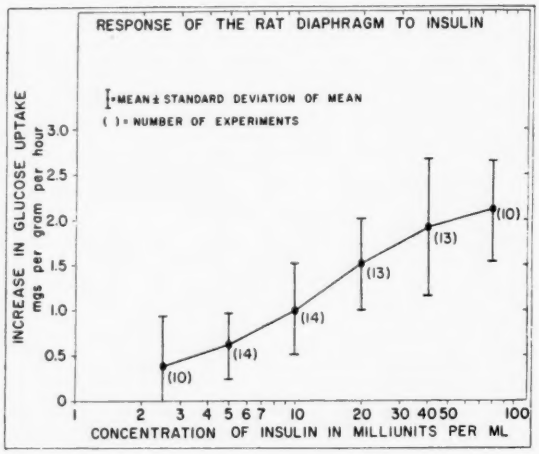


FIG. 7. Log-dose response curve of the rat hemidiaphragm to standard concentrations of insulin.

cases insulin-like activity of the serum at sixty minutes was below the sensitivity of the assay procedure. At fifty units per kg. body weight, insulin-like activity remained above the upper limits of the dose response curve between thirty and sixty minutes after injection.

Seven normal rabbits were given an intravenous injection of fifteen units of crystalline zinc insulin per kg. body weight. The effect of sera obtained from the rabbits on the glucose uptake of the rat hemidiaphragm is shown in figure 8. The rat hemidiaphragms were incubated in the sera obtained from the rabbits for two minutes at

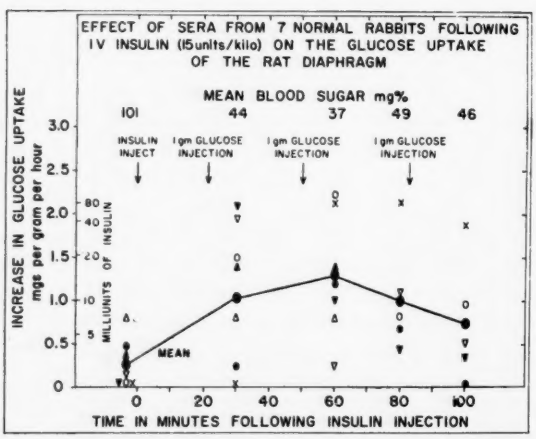

EFFECT OF SERA FROM 7 NORMAL RABBITS FOLLOWING IV INSULIN (15units/kilo) ON THE GLUCOSE UPTAKE OF THE RAT DIAPHRAGM
MEAN BLOOD SUGAR mg%
101
44
37
49
46
INSULIN INJECT
1 gm GLUCOSE INJECTION
1 gm GLUCOSE INJECTION
1 gm GLUCOSE INJECTION
INCREASE IN GLUCOSE UPTAKE mgs per gram per hour
MILLIUNITS OF INSULIN
MEAN
TIME IN MINUTES FOLLOWING INSULIN INJECTION

FIG. 8. The effect of the sera from each rabbit on the glucose uptake of the diaphragm is represented by a different symbol. The mean value for each time interval is shown by the closed circle.

37° C. as stated in the methods. The results are expressed as the absolute gain of glucose in mg. per gram hour of the treated hemidiaphragms over the controls. The comparable concentration of insulin producing the same effect in the bio-assay is shown on a scale alongside the glucose uptake. The results were expressed in this manner to avoid the inference that insulin alone was being measured in these sera. The sera obtained from the rabbits before injection of the insulin increased the glucose uptake of the diaphragms to an average of 0.25 mg. per gram hour. This is equivalent to an insulin effect of less than 2.5 mu. per ml. of serum. At thirty and sixty minutes following injection, there is considerable individual variation in the glucose uptake with an average gain of 1.01 and 1.29 mg. per gram per hour respectively. Equivalent effects are produced by insulin in concentrations of ten and fifteen milliunits per ml. At eighty minutes the response is more uniform, and there is a decrease in the average glucose uptake to 1.05 mg. per gram hour, equivalent to an insulin effect of eleven milliunits per ml. The average response in glucose uptake decreased further at 100 minutes to 0.75 mg. per gram hour, equivalent to an insulin effect of 6.0 mu. per ml. In the sera taken at 140 minutes from three of these rabbits the insulin-like effect had returned to the pre-injection level. In these experiments the rabbits received intravenous glucose at twenty, forty, and sixty minutes in order to prevent hypoglycemia. However, the blood sugar figures were low and although no hypoglycemic signs occurred, this may be a factor influencing the results.

From the previously determined space of distribution and the rates of disappearance for TCA precipitation and electrophoresis, a calculation has been made of the theoretical concentrations of insulin present in the serum from 0 to 140 minutes after the injection of insulin in the dose of fifteen units per kg. body weight. These concentrations have been compared with the insulin-like activity recovered by bio-assay (figure 9). The line representing the TCA concentrations is outside the standard deviations about the mean of the bio-assay levels, except at sixty minutes. There is little correlation between the electrophoretic and the bio-assay concentrations at thirty minutes. Between sixty and 140 minutes the concentrations derived from the electrophoresis results are within the standard deviations for the levels obtained by bio-assay.

The biologic activity of one lot of radioactive insulin was measured by its effect on the rat hemidiaphragm, using the mean of three diaphragms. At a concentration of forty milliunits per ml., the activity was 65 per cent

of that of the glucagon-free insulin standard at this concentration.

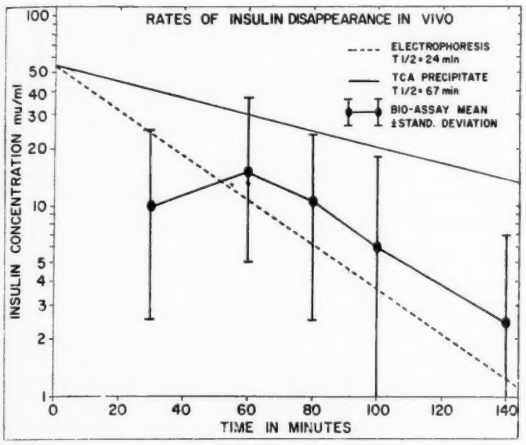


FIG. 9. Rates of insulin disappearance in vivo comparing trichloroacetic acid precipitation, paper electrophoresis and the bio-assay, at a dose level of fifteen units of crystalline zinc insulin per kg. body weight.

DISCUSSION

The study of insulin metabolism with insulin I^{131} is based on the assumptions that the radioactive insulin is unaltered by the labeling process and that it is representative of endogenous insulin. An accepted method of measuring radioactive insulin in biological fluids has been the precipitation of radioactivity by the addition of trichloroacetic acid. Recently, however, Berson and his associates have found that a variable portion of insulin is physically altered by the labeling process.[3] This is demonstrated by the behavior of insulin I^{131} when studied by electrophoresis. Under these conditions, unaltered insulin remains adsorbed to the paper strip at the point of application, whereas the altered insulin moves at a rate approximately equal to that of albumin whether or not plasma proteins are present. This altered fraction may represent as much as 25 per cent of the total radioactivity. The technics of TCA precipitation and electrophoresis were compared in a study of the rates of disappearance of insulin I^{131} from the plasma of rabbits. The radioactive insulin was administered intravenously. The biological half life of insulin I^{131} as determined by TCA precipitation was found to be much longer than that determined by measurement of the radioactivity adsorbed at the point of application in the electrophoretic technic. The prolonged half life of the radioactivity of the TCA precipitate was shown to be due to the persistence in the circulation of a physically altered fraction of the insulin I^{131}. Similar physical alterations in the insulin molecule have been produced by external irradiation with X rays in doses comparable to those given by I^{131}.[11] Chemical analysis of the insulin irradiated by either X ray or I^{131} has demonstrated that the disulfide bonds of insulin are separated and reduced to the sulfhydryl form during irradiation.[12]

In the studies reported here, rates of disappearance of insulin I^{131} were also measured in the rabbit following intravenous administration. The mean biological half life of the TCA precipitate from the plasma was sixty-seven minutes, whereas that of the radioactivity adsorbed at origin in the electrophoretic technic was twenty-four minutes. The difference found between the absolute counts of the radioactivity measured in these two methods was relatively constant throughout the period of study (figure 3). The radioactive fraction was found to migrate with the plasma proteins, when studied by electrophoresis (figures 1, 6), and persisted, with no measurable biological decay, after disappearance of the unaltered insulin fraction from the plasma (figure 3). It was this recirculating altered fraction which accounted for the prolonged biological half life of the TCA precipitate.

Bio-assay of the insulin-like activity of sera obtained from rabbits, following intravenous injection of unlabeled insulin, was made using the rat hemidiaphragm technic. There was a wide individual variation in the level of insulin found at thirty and sixty minutes. This may have been due to the presence of insulin antagonists in some sera samples. From sixty to 140 minutes following injection of insulin there was a gradual decrease in the mean level of insulin in the serum with a return to the pre-injection level at about 140 minutes.

The volume of distribution of insulin I^{131} and the rates of disappearance of insulin I^{131}, as determined by TCA precipitation and electrophoresis, were used in the calculation of the theoretical concentration of insulin present in the serum. From sixty to 140 minutes following injection of insulin there was good correlation between the levels of insulin measured by the bio-assay and the theoretical concentration of insulin I^{131} as calculated from the previous electrophoretic study of sera. At 140 minutes the insulin level had returned to the pre-injection level as measured by bio-assay, and the theoretical concentration of insulin as determined by the electrophoretic technic was similar to that of the bio-assay. At this time the insulin concentration calculated by the TCA precipitation technic was persistently high and showed poor correlation with the bio-assay of un-

labeled insulin. The rate of disappearance of insulin I^{131} from the plasma as measured by the technic of TCA precipitation is much slower than the disappearance of unaltered insulin as determined by bio-assay. This implies that the residual recirculating radioactivity, equivalent to the altered insulin fraction, is biologically inactive.

The observation that a standard solution of insulin I^{131} had less effect on the glucose uptake of the diaphragm than an equivalent concentration of unlabeled insulin also suggests that biological activity is diminished by the labeling process. However, further comparisons of the labeled and unlabeled insulin at various concentrations will be necessary to confirm this finding.

Thus, biological inactivation of insulin I^{131} may occur without the separation of the radioactive label. This places a limitation on the use of insulin I^{131} as a reliable tracer of insulin metabolism. Some of these limitations are overcome, however, by the use of the electrophoretic technic or by more refined method of iodination with I^{131}.[13, 14]

SUMMARY

Insulin, labeled with radioactive iodine (I^{131}), was given to normal rabbits by intravenous injection. The rate of disappearance of the radioactivity from the plasma was studied by two technics. In the first, the radioactivity in the plasma was precipitated with trichloroacetic acid; whereas in the second, the insulin I^{131} was separated from the plasma by electrophoresis. The rate of disappearance of the radioactivity was found to be much slower by the technic of trichloroacetic acid precipitation than by that of plasma electrophoresis. The biological half life of the radioactivity in the first method was sixty-seven minutes, whereas in the second it was twenty-four minutes. The longer biological half life of the radioactivity precipitated by trichloroacetic acid was due to persistence of a portion of the radioactivity migrating with the plasma proteins.

The rate of disappearance of unlabeled insulin from the plasma of rabbits was also studied by means of a bio-assay. This rate of disappearance showed good correlation with that of the disappearance of radioactive insulin found by electrophoresis. Conversely, the rate of disappearance as determined by bio-assay did not correlate with that found by trichloroacetic acid precipitation. The theoretical levels of insulin predicted by the electrophoretic technic from sixty to 140 minutes following administration of insulin were similar to the levels of insulin found by bio-assay. These results do not support the assumption that the trichloroacetic acid precipitate of radioactive insulin in serum represents only intact, biologically active insulin. However, the method for the separation of insulin I^{131} from plasma employing electrophoresis appears to be accurate.

SUMMARIO IN INTERLINGUA

Un Comparation Del Destino De Insulina In Le Sero, Sin E Con Marcage Per I^{131}

Insulina marcate con iodo radioactive (I^{131}) esseva administrate a conilios normal per injection intravenose. Le rapiditate del disparition del radioactivitate ab le plasma esseva studiate per medio de duo technicas. In le prime, le radioactivitate in le plasma esseva precipitate per acido trichloroacetic. In le secunde, le insulina a I^{131} esseva separate ab le plasma per electrophorese. Le valor trovate pro le rapiditate del disparition del radioactivitate per medio del technica a precipitation trichloroacetic esseva multo plus alte que illo trovate per medio del technica a electrophorese del plasma. Le medie vita biologic del radioactivitate secundo le prime methodo esseva sexanta-septe minutas; secundo le secunde, vinti-quatro minutas. Le plus longe medie vita biologic del radioactivitate precipitate per acido trichloroacetic esseva causate per le persistentia de un portion del radioactivitate migrante con le proteinas del plasma.

Le rapiditate del disparition de non-marcate insulina ab le plasma de conilios esseva etiam studiate per medio de un bio-essayo. Le valores assi trovate monstrava un bon grado de correlation con le valores trovate pro le rapiditate del disparition del insulina radioactive per medio del technica electrophoretic. Del altere latere, le rapiditate del disparition determinate per bio-essayage non se monstrava in correlation con le valores determinate per precipitation a acido trichloroacetic. Le nivellos theoric de insulina predicite super le base del methodo electrophoretic pro sexanta a quaranta minutas post le administration de insulina esseva simile al nivellos trovate per bio-essayage. Iste resultatos non supporta le supposition que le precipitato de insulina radioactive effectuate in le sero per acido trichloroacetic representa solmente insulina que es intacte e biologicamente active. Tamen, le methodo de separar insulina a I^{131} ab le plasma per medio de electrophorese es apparentemente accurate.

ACKNOWLEDGMENT

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DISCUSSION

FRANCIS D. W. LUKENS, M.D., (*Philadelphia*): It is a pleasure to hear this critical comparison of different methods of assaying or appraising the presence of insulin. We have run into a similar problem in Philadelphia, in which an immunologic method of measuring insulin has failed to agree with Dr. Vallance-Owen's bio-assay. The fact that two such methods are being critically compared by the Baltimore group is an encouraging thing for the methods of tomorrow.

Galactose Utilization in Sucklings

More critical evaluation of galactose tolerance revealed that suckling rats can utilize galactose completely when it is present in no larger proportion than 5 per cent of the total diet by dry weight. If fat or protein rather than glucose is present as a high per cent of the diet, the tolerance can be elevated to approximately 10 per cent, the criterion for tolerance being the appearance of galactose in the urine. Young rats fed an artificial mixture of 44 per cent fat, 39 per cent casein, 5 per cent salt mixture, and 12 per cent lactose nevertheless contained 10 per cent less total liver lipid than did rats fed a similar diet except for the substitution of glucose for lactose. Unfortunately no data from the above experiments relate the absolute rather than relative amounts of galactose in the diet to the changes reported.

When comparison is made of the lactose content of rat's milk with that of other animals, it is found that the former contains only about 9 per cent lactose, whereas cow's milk contains about 38 per cent lactose and human milk contains almost 57 per cent. This may indicate that galactose tolerance differs widely among mammalian sucklings. Apparently, suckling as well as adult rats differ from human and bovine species in that they lack the capacity to utilize galactose as a major source of energy (galactosemia occurs only very rarely in human infants). Indeed, it was reported by Scheunert and Sommer that when galactose is withheld from 40-gm. suckling rats, development takes place readily in the presence of glucose. Thus the role of this sugar in development remains obscure. Perhaps other species are more dependent on its presence in the diet than is the rat. It has been reported that milk sugars aid in the absorption of calcium, a finding which is supported by the present studies on mineral deposition but seems to be contradicted by the X-ray studies of P. Handler (J. Nutrition *33*:221, 1947). It is further suggested that galactose is involved in the development of the central nervous system, in which there is intensive activity in many species during the first few postpartum days.

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Paper Electrophoresis of Serum Proteins in Diabetic Patients

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The serum protein patterns of diabetic individuals have been investigated intensively in the past few years. Interest in such studies began in 1949 with the histological demonstration of mucopolysaccharides in the vascular lesions of diabetic patients,[1, 2] and with the finding that changes in serum lipoprotein patterns may be associated with the lesions of atherosclerosis.[3] The development of the technic of paper electrophoresis has made it feasible to carry out detailed studies on serum protein patterns in a wide range of clinical disorders.

Although a number of studies have been made of the electrophoretic pattern of the serum proteins in diabetics,[4–15] these earlier investigations were made on small groups of patients and yielded conflicting data. Furthermore, in certain of these studies the clinical status of the patient was not clearly defined, particularly with regard to vascular complications. It seemed that a study of a large group of diabetic patients, with careful evaluation of clinical status, might resolve some of the disparate results reported. Therefore, investigation of such a group, with and without vascular complications, was undertaken in an attempt to provide further correlation of changes occurring in serum protein fractions with those occurring in the vascular system.

MATERIALS AND METHODS

The paper electrophoresis studies were done using, in slightly modified form, the technic of Kunkel and Tiselius.[16] The electrophoretic procedure was carried out on 0.005 ml. serum applied to Whatman 3MM filter paper and used with a sodium veronal-sodium acetate buffer of ionic strength 0.1, *p*H of 8.6.[17] This buffer has given the best separations of the α-globulins in this laboratory and in at least one other.[17] The current was approximately 0.4 to 0.5 ma. per cm. width of paper and electrophoresis was carried out for about fourteen hours at room temperature (20 to 22° C.).* Ninety-five per cent of the strips were stained with Wool Black (Harleco†). The strips were rendered translucent in a mixture of α-bromonaphthalene and white mineral oil.[17] They were then sealed between cellophane tapes and electrophoretic curves determined using a photovolt densitometer (Model No. 525, 610 mμ). The relative percentages of the individual serum protein fractions were calculated from the areas measured with a planimeter. Total serum protein was determined by the Biuret method, and absolute values for each fraction were obtained by multiplying the relative percentages by the total protein content of the serum.

Initially, determinations were made using a buffer of ionic strength 0.05 and staining the paper strips with bromphenol blue. The ionic strength of the buffer was later changed to 0.1 in order to separate better the α-globulins from each other and from albumin.[17] The change to the dye Wool Black was made for two reasons: 1) Wool Black appears to be more sensitive to small changes in protein concentration, and 2) if the serum albumin content of the sample exceeded 3.6 gm. per cent, strips stained with bromphenol blue and read on this densitometer may not yield accurate values for either albumin or γ-globulin. The use of very small amounts of sera would eliminate this, but would introduce a greater sampling error. A detailed discussion of the comparison and validity of the two technics and of other aspects of the method used is reported by Tuller.[19]

Thus it was decided early in the study to use Wool

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*The voltage was not recorded or used as a means of regulation in the method given here, since the procedure had been arrived at empirically and found to be controllable by achieving the current density described. Subsequent work with the horizontal open strip method, however, has been based on voltage per cm. length of free paper.[18]

†Although this is now no longer available, Amido Black 10B (A. H. Thomas) has been shown to be equivalent in purity and results.[19]

Black instead of bromphenol blue. In those instances in which the original determinations were made with bromphenol blue, new separations of the serum proteins were prepared, the strips stained with Wool Black, and compared to the original strips. In about 5 per cent of the cases this comparison was not possible. However, all of these latter individuals had low values for both total protein and serum albumin, and it is probable that the initial staining gave accurate results.

Most studies, including this one, are done on single samples taken from individuals. Because serum protein patterns of a disturbed metabolic state might vary more with time than would those of normal individuals, the serum protein distribution was determined at intervals covering a period of several months in three healthy nondiabetic individuals and in three hospitalized diabetic patients. There were no important variations in the values for any fraction with the passage of time, even in one of the diabetic subjects who had an extremely poor prognosis. Patients with keto-acidosis were excluded, and in a majority of cases the blood samples were taken after control of the diabetes had been established under hospital conditions. Therefore, any changes from normal which appear in the pattern of the serum proteins of any diabetic individual should be due first to the metabolic disorder of long-term diabetes, and secondly might be connected with the presence of the vascular complications.

There is, at present, no evidence that there is any difference in dye uptake between any given fraction of normal sera and the corresponding fraction of pathologic sera. However, it is known that a variety of diseases affect the serum protein patterns.[20] It is, therefore, imperative that a careful clinical evaluation of all patients studied be made, since conflicting results may be obtained, if complications of a nondiabetic nature are present in the diabetic individual.

Clinical Material. The sera of 120 diabetic patients were examined. All patients were eliminated from this report if they had complicating conditions such as multiple myeloma, infectious diseases, hemochromatosis, keto-acidosis or diabetic coma, gangrene, or osteomyelitis. Of the seventy-nine remaining cases, sixty-four had developed diabetes prior to the age of thirty. All were in good nutritional status. The seventy-nine individuals were grouped as follows:

A. No clinical evidence of diabetic complications.

B. Neuropathic changes only.

C. Retinopathy but no clinical evidence of renal involvement.

D. Retinopathy and nephropathy without neuropathy.

E. Retinopathy, nephropathy and neuropathy (diabetic "triopathy"[21]). Retinopathy and nephropathy were diagnosed according to the criteria used by the Joslin Clinic.[22] A diagnosis of neuropathy was based on a clear history or finding of involvement of the central nervous system. The neuropathies included peripheral neuritis, "neuropathic foot," diabetic diarrhea, paresis of the urinary bladder, and postural hypotension.

RESULTS

Table 1 presents the clinical data for the five groups of patients. The age at onset was comparable in the five groups with the exception of Group B (neuropathic changes only), where the age at onset was somewhat later. The duration of diabetes was less in those patients with no complications, as would be expected, and also in the patients with neuropathy. However, 60 per cent of the individuals in both these groups (A and B) had had diabetes for more than ten years. The daily insulin requirement was somewhat less in Groups D and E, patients with the most severe complications.

The data obtained from paper electrophoretic studies of the sera of the five groups of patients and from six normal controls are given in table 2. The data for the six controls are closely comparable to those obtained on twenty nondiabetic controls studied in this laboratory with the horizontal open-strip method,[23] and also agree well with the values obtained by Bogdanowicz, Osinski and Stein[12] in twenty-five nondiabetic subjects.

The most accurate way of presenting values for each protein fraction is in grams per cent (absolute), but in order to compare the results with those of other investigators it is also necessary to present relative percentages; therefore, both types of data have been given in tables 2 and 3.

Statistical analysis of the data in table 2 shows that the α_1- and γ-globulin values are not significantly altered in any of the diabetic groups when compared to those for the normal controls.* Since no significant difference was found in the distribution of the protein fractions between diabetic patients without complications (A) and diabetics with only neuropathy (B), they were combined as Group AB. Similarly, the data from Group D (retinopathy and nephropathy) were compared with the data from Group E ("triopathy": retinopathy, nephropathy and neuropathy), and as there was no statistically significant difference in the serum protein fractions between these groups, they were combined as Group DE.

*Unless otherwise indicated, p is less than or equals 0.01 whenever the term "significant" is used. The t-test was used in calculating comparisons between groups.

TABLE 1

Clinical data on diabetic subjects

		Diabetic groups*				
		A	B	C	D	E
Number in group		28	7	7	11	26
Age at time of study, years	Average	28	38	36	38	40
	Range	(12-55)	(24-51)	(26-42)	(25-57)	(23-75)
Age at onset of diabetes, years	Average	18	27	18	19	20
	Range	(1-55)	(18-36)	(3-32)	(1-45)	(2-64)
Duration of diabetes, years	Average	10	10	18	19	19
	Range	(0.5-29)	(0.5-27)	(10-30)	(12-24)	(11-30)
Daily insulin dose, units	Average	56	63	55	47	42
	Range	(12-112)	(14-100)	(34-72)	(20-80)	(20-80)

*A=Without complications B=With neuropathy only C=With retinopathy only
D=With retinopathy and nephropathy E=With "triopathy" (neuropathy, retinopathy and nephropathy)

TABLE 2

The serum proteins of nondiabetic and diabetic individuals

				Protein fractions				
					Globulins			
Group*		No. of subjects	Total protein per cent S.D.	Albumin per cent S.D.	α_1 per cent S.D.	α_2 per cent S.D.	β per cent S.D.	γ per cent S.D.
				Components expressed as percentages of total planimeter areas				
Nondiabetic		6		56.9±2.2	4.9±0.9	8.7±2.0	13.3±1.8	16.2±2.2
Diabetic:	A	28		53.4±6.0	4.4±1.7	11.5±2.2	14.9±2.5	16.1±3.7
Diabetic:	B	7		50.4±4.0	5.1±1.3	11.3±2.4	16.8±1.4	16.4±2.2
Diabetic:	C	7		50.7±2.7	5.1±0.9	12.4±1.8	15.2±2.9	16.6±2.3
Diabetic:	D	11		43.3±7.4	5.8±1.6	16.7±4.4	16.7±2.5	18.1±3.0
Diabetic:	E	26		42.5±6.6	6.0±1.7	16.8±3.6	17.3±2.6	17.5±4.6
				Components expressed in grams per cent				
Nondiabetic		6	6.9±0.5	3.9±0.24	0.34±0.05	0.60±0.12	0.92±0.15	1.1±0.23
Diabetic:	A	22	6.4±0.8	3.4±0.57	0.29±0.09	0.71±0.14	0.96±0.17	1.0±0.25
Diabetic:	B	5	6.3±0.2	3.1±0.23	0.33±0.09	0.76±0.14	1.04±0.08	1.1±0.15
Diabetic:	C	5	6.9±0.7	3.4±0.46	0.33±0.06	0.82±0.14	1.05±0.18	1.1±0.13
Diabetic:	D	11	6.3±1.0	2.7±0.69	0.36±0.11	1.04±0.28	1.04±0.19	1.1±0.24
Diabetic:	E	21	5.7±0.9	2.8±0.62	0.33±0.08	0.92±0.19	0.98±0.15	1.0±0.29

*For criteria of classification see footnote of table 1.

Table 3 presents the mean ratios and standard deviations for the nondiabetic group and the regrouped diabetics. The data show significantly decreasing values for both relative and absolute amounts of serum albumin and significantly increasing values for the α_2-globulin fraction. However, the only significant change in the values for the β-globulin fraction was the increased per cent of this component in the serum of individuals in Group DE as compared to the nondiabetic group. Table 4 shows the statistical analysis of the comparison of the data for regrouped diabetics and that for the nondiabetic groups.

Relationship of Serum Proteins and Retinitis Proliferans. Since there has been some interest in the possibility of a difference in the serum protein patterns in individuals with diabetes and nephropathy with and without retinitis proliferans, Group DE was subdivided into twenty-three patients with and fourteen without this latter complication. The only difference found in the serum protein patterns of the two groups was an increased value for α_2-globulin in patients with retinitis proliferans. The *p*-value for the increase in both relative and absolute values for α_2-globulin was 0.08, which approaches borderline significance. Thus, this increase in

TABLE 3

The serum proteins of nondiabetic and diabetic individuals*

Group	Total protein per cent	S.D.	Protein fractions: Albumin per cent	S.D.	Globulins: α_1 per cent	S.D.	α_2 per cent	S.D.	β per cent	S.D.	γ per cent	S.D.
			Components expressed as percentages of total planimeter areas									
Nondiabetic			56.9	±2.2	4.9	±0.9	8.7	±2.0	13.3	±1.8	16.2	±2.2
Diabetic: AB			52.8	±5.6	4.5	±1.3	11.5	±2.3	15.3	±2.4	16.1	±3.3
Diabetic: C			50.7	±2.7	5.1	±0.9	12.4	±1.8	15.2	±2.9	16.6	±2.3
Diabetic: DE			42.8	±5.9	6.0	±1.5	16.8	±3.9	17.1	±2.7	17.7	±4.0
			Components expressed in grams per cent									
Nondiabetic	6.9	±0.5	3.9	±0.24	0.34	±0.05	0.60	±0.12	0.92	±0.15	1.1	±0.23
Diabetic: AB	6.4	±0.6	3.3	±0.50	0.30	±0.09	0.72	±0.14	0.98	±0.14	1.0	±0.23
Diabetic: C	6.9	±0.7	3.3	±0.46	0.33	±0.06	0.82	±0.14	1.05	±0.18	1.1	±0.13
Diabetic: DE	5.9	±0.9	2.5	±0.70	0.34	±0.10	0.96	±0.24	1.00	±0.15	1.0	±0.28

*Compilation of data showing means determined by combining the original data of Groups AB and that of Groups DE. Designation of original groups is given in footnote of table 1.

TABLE 4

Statistical analysis of the data from nondiabetic and diabetic groups (p values)

Groups compared	Serum protein fractions: Albumin	α_2	β
	Relative per cent		
Nondiabetic–AB*	N.S.†	<0.01	N.S.
Nondiabetic–C	<0.01	<0.01	N.S.
Nondiabetic–DE	<0.001	<0.01	<0.01
	Grams per cent		
Nondiabetic–AB	<0.02	N.S.	N.S.
Nondiabetic–C	<0.05	<0.02	N.S.
Nondiabetic–DE	<0.001	<0.001	N.S.

*Designation of original diabetic groups is given in footnote of table 1.
†N.S.=Not significant

the values might prove to be significant in larger groups.

The age at onset of diabetes may have influenced these findings. The average age at onset of the disease was fifteen years in the patients with retinitis proliferans, but was twenty-seven years in the other patients. The duration of disease was the same in both groups (approximately nineteen years). No comparative estimate of the severity of the renal disease was made in any of these individuals, but it is possible that it was more advanced in those with retinitis proliferans.

Relationship Between Elevated α_2-Globulin and Prognosis. Immediate examination of the individual values for serum protein fractions showed that five patients in Group DE had extremely high relative and absolute amounts of α_2-globulin. Also, the relative percentages of the β-globulin fractions for these five patients were somewhat elevated. Investigation of these patients revealed that all were comparatively young and had had their diabetes for from seventeen to twenty-nine years. Clinically, their prognosis was poor, and follow-up studies confirmed this since all died within forty-eight hours to one year after the analysis of serum protein had been made. Five additional patients, all in Group DE, were found also to have died within one year of the study. The clinical data and the distribution of the plasma proteins in these ten patients are presented in table 5.

Of the above ten patients, the immediate cause of death was not known in two. One patient who showed a very high α_2-globulin content died of a myocardial infarction and the other seven died in uremia. Although an extremely high relative and absolute value for the α_2-globulin fraction appears to correlate with a poor prognosis, the converse is not necessarily true, since an equal number of individuals with lower values succumbed within the same period of time (table 5).

DISCUSSION

These results confirm the consistent findings of others[10–13, 15] on small or specialized groups of diabetic patients and show values which were decreased for the albumin fraction and elevated for the α_2-globulin in the presence of vascular complications. This study also has demonstrated a tendency for these serum protein changes to be present *before* there is clinical evidence of vascular damage. There were no changes in the α_1- and γ-globu-

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TABLE 5

Serum protein patterns on patients dying within two years of protein analysis

Case no.	Age	Duration of diabetes	Total protein grams per cent	Protein fractions: Albumin Per cent of total area	Albumin Grams per cent	Globulins: α_1 Per cent of total area	α_1 Grams per cent	α_2 Per cent of total area	α_2 Grams per cent	β Per cent of total area	β Grams per cent	γ Per cent of total area	γ Grams per cent
1	39	26	3.5	32.0	1.1	8.9	0.31	27.2	0.95	19.0	0.67	12.7	0.45
2	41	17	4.8	31.3	1.8	6.9	0.33	21.3	1.02	19.7	0.95	20.8	1.00
3	34	33	5.1	33.1	1.7	5.4	0.27	26.8	1.37	18.5	0.94	16.2	0.83
4	36	20	5.5	36.3	2.6	5.0	0.28	23.0	1.26	17.5	0.96	18.2	1.00
5	47	29	5.7	29.0	1.6	6.3	0.35	20.4	1.16	14.8	0.85	29.6	1.70
6	33	23	—	33.2	—	6.6	—	20.5	—	18.0	—	21.7	—
7	23	20	5.2	43.4	2.4	10.4	0.52	18.6	0.96	18.3	0.94	9.3	0.48
9	53	15	5.3	41.6	2.2	6.0	0.32	18.7	0.99	15.0	0.79	18.7	0.99
11	75	11	—	41.5	—	7.8	—	15.6	—	15.9	—	15.3	—
12	40	12	5.4	42.3	2.3	7.4	0.40	11.8	0.84	16.6	0.90	21.8	1.18

lin fractions, although other workers have reported changes in the latter component.[8, 13–15] The results reported here are suggestive of an increase in the relative percentages for the β-globulin fraction, although this increase is not statistically significant except in those patients with well advanced vascular complications (Group DE). There was no suggestion of this change in the absolute values.

The data presented here do not, unfortunately, resolve the controversy concerning the values for the β- and γ-globulin fractions in diabetic individuals with retinopathy and nephropathy. Much of this controversy may be due to differences in technics. Reports based on measurements made by free electrophoresis indicate an elevation of the relative percentages of the β-globulin fraction.[4–8] These increased values are probably due to refractive index variations because of the large amounts of lipoproteins present in the sera of diabetic patients with vascular complications.[24] On the other hand, investigators who used the technic of paper electrophoresis have reported an increase in the percentages for the β-globulins, while reporting no change[12] or a decrease[15] in the γ-globulin fraction. Since Amido Black 10B and bromphenol blue only stain the protein moiety, and since the amount of protein material in the lipoproteins is about 25 to 35 per cent of the total molecule, any increases in lipoproteins (such as up to 300 mg. per cent) in the sera of diabetic individuals probably will not produce detectable increases in the protein moiety of the β-globulin fraction. However, large amounts of lipoproteins tend to make it more difficult to resolve the β- and γ-globulin fractions. Therefore, it is possible that the disparity in results concerning these fractions is due to problems of interpreting the electrophorerograms.

The present study indicates that there is a decrease in serum albumin in diabetic patients without clinically demonstrable nephropathy, but that it does not clarify the origin or possible significance of such a decrease. The marked fall in the amount of serum albumin in the diabetic with nephropathy is undoubtedly due to impaired renal function. The change in albumin values in the other diabetic subjects may be due to early subclinical vascular change. An elevation of the blood urea nitrogen (one of the criteria of renal damage) does not occur until 75 per cent of the nephrons have been destroyed,[25] although albuminuria may appear earlier. Also, the work of Ditzel[26] demonstrates that changes in the smaller vessels take place early in the diabetic state, accompanied by the exudation of plasma fluid into the perivascular tissue. Therefore, renal vascular changes may exist undetected in those diabetics with clinically demonstrable retinopathy, and this may be primarily responsible for the lowered values of serum albumin seen in these patients. In patients with no clinically detectable retinopathy or nephropathy, however, changes of the type demonstrated by Ditzel may be the paramount reason for the initial decrease in albumin. Also, Young and Webber[9] have suggested that hepatic function should be studied in many pathological states, since the origin of albumin appears to be the liver. Although it is possible that there is some hepatic dysfunction in the patient who is not in obvious poor control as shown by keto-acidosis, clinical studies have shown such dysfunction to be uncommon in diabetic individuals apart

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from frank liver diseases.[27] Thus, in diabetic individuals without clinical nephropathy, both the origin and the significance of the decrease in serum albumin values are unknown.

The increased α_2-globulin values are very striking and may be of importance. Although there is a significant increase in the amount of the α_2-globulin fraction in the *presence* of retinopathy and nephropathy, increased values were also observed in patients *without* these complications. This confirms the work of Bogdanowicz, Osinski and Stein,[12] who also studied a comparable group of individuals with early onset of diabetes. Little is known about the origin of the α_2-globulins, but Young and Webber[9] have suggested a possible relationship to disturbances in bone metabolism. The progressive, marked, and selective increase in this fraction of the plasma proteins suggests that it may be of significance in the pathogenesis of the retinal and renal vascular lesions of diabetes. As has been demonstrated histochemically, these lesions contain large amounts of mucopolysaccharides[1, 2] and a large proportion of protein-bound carbohydrates are known to be bound to the α_2- fraction.[28] On the other hand, this increase cannot be regarded as specific for diabetes, as elevations of α_2-globulin have been reported in acute inflammatory diseases, in acute tuberculosis and in certain malignant diseases.[9, 20] There is no evidence as to whether the increase in the different clinical conditions is due to a common stimulus, or occurs in response to different pathological or metabolic stimuli. A study of the subfractions of the α_2-globulin component might clarify this problem by showing whether the increase in amount is due to the same or to different subfractions in the various disease states.

The possible relationship between vascular complications and variations in the serum protein pattern has been considered above. However, other variables may play a part in these changes. For example, although neuropathy appears to have no effect on the serum protein pattern in the presence of the vascular complications, limited ultracentrifugal studies on lipoproteins[29] indicate that some relationship may be present. It is also possible that the increase in the relative percentage of the β-globulin fraction in Group B (neuropathy alone) may be related to changes in lipoproteins. However, in order to study individuals exhibiting symptoms of neuropathy only, it was necessary to select individuals whose average age at onset of diabetes was greater than that of those diabetics without any complications.

Subsequent investigation has tended to confirm the impression that, whatever the cause of the central nervous system disorder in diabetes, there is a concomitant small change in the serum proteins or their conjugates.

In order to consider the duration of diabetes as an independent variable and its possible effect on the serum protein pattern, it is necessary to consider only the data from Group A (no complications). No statistical correlation of protein values with duration could be made, since there was considerable scatter of the data in this group of patients and the durations of the disease were not well distributed. However, the data gave the impression that the α_2-globulin values became progressively elevated with the increase of the duration of diabetes. A subsequent study in this laboratory also confirmed this impression.[23]

SUMMARY

1. The serum protein patterns of seventy-nine diabetic patients in various stages of the disease were determined by use of paper electrophoresis analysis.

2. Previous results on small groups of diabetics with vascular complications were confirmed: namely, a decrease in the serum albumin, no change in the α_1-globulin fraction and an increase in the α_2-globulin fraction. The controversy regarding possible changes in the β- or γ-globulin fractions was not resolved.

3. Decreases in the albumin and increases in the α_2-globulin fractions also were found, though of lesser degree, in diabetic subjects without complications.

4. The duration of diabetes and the progression of the vascular complications are two factors which appear to affect the serum protein pattern.

SUMMARIO IN INTERLINGUA

Electrophorese a Papiro del Proteinas Seral in Patientes Diabetic

1. Le configurationes del proteinas seral de septantanove patientes diabetic in varie stadios del morbo esseva determinate per medio de electrophorese a papiro.

2. Le resultatos previemente obtenite con pauco numerose gruppos de diabeticos con complicationes vascular esseva confirmate. Iste resultatos esseva un reduction del albumina seral, nulle alteration in le fraction globulina alpha-1, e un augmento in le fraction globulina alpha-2. Le controversia relative a possibile alterationes del fractiones globulina beta e gamma non esseva resolvite.

3. Reductiones de albumina e augmentos del fraction globulina alpha-2 esseva constatate etiam in diabeticos sin complicationes, sed a grados minus pronunciate.

4. Le duration del diabete e le progression del complicationes vascular es duo factores que affice apparentemente le configuration del proteinas seral.

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Information from all available sources indicates a rising incidence of acute myocardial infarction among white individuals in the United States and the data from the autopsies studied by the group at Washington University tend to confirm this rise. The over-all incidence of acute myocardial infarction among the Barnes autopsies was twenty-nine times as high in the decade 1945 to 1954 as it was in the decade 1910 to 1919. The rise occurred in all age groups of both sexes and was not simply a result of an aging population. No evidence was found to indicate that the rise in incidence could be accounted for by a rise in the incidence of diabetes mellitus, hypertension, and obesity. It seems likely that some changing factor(s) in our civilization is responsible and it is remarkable that its effect has been almost exclusively confined to members of the white race.

From "Fatal Acute Myocardial Infarction: Sex, Race, Diabetes and Other Factors," by Wilbur A. Thomas, M.D., in *Nutrition Reviews*: Vol. 15, No. 4, April 1957, pp. 97-101.

Oral Hypoglycemic Agents

Panel Discussion

Arthur R. Colwell, Sr., M.D., Chicago
Moderator

Henry Dolger, M.D.
New York City

Rachmiel Levine, M.D.
Chicago

Garfield G. Duncan, M.D.
Philadelphia

Mary A. Root, Ph.D.
Indianapolis

PRESIDENT REED: The panel will discuss some of the new oral hypoglycemic agents. I take this opportunity to introduce Dr. Arthur R. Colwell, Sr., of Northwestern University Medical School, who will moderate the program.

DR. COLWELL: The release is imminent for general use of a sulfonamide compound which, when given by mouth, has unquestioned ability to lower blood sugar values both in normal and in many diabetic subjects. All are properly interested in several major points. How does the substance act? How should it be used in treatment? Are there risks in its use? What is its relationship, if any, to insulin? This panel will endeavor to provide answers to these and other questions. If there is disagreement among us it will reflect the difficulties which have been encountered in trying to find reliable answers to many important questions, particularly concerning mechanisms of action.

A great deal of experimental and clinical data has been accumulated during the last two years or so concerning the sulfonylurea compounds. The experts at my side are well-informed concerning them and have contributed generously to their study. To start the discussion, I will ask each of the panelists to give us a brief orientation on certain aspects of the subject. For the most part, this will concern the sulfonylurea compounds, and specifically tolbutamide, which is marketed under the trade name of Orinase, the only compound available now clinically in this country.

Dr. Root, will you give a brief historical background and identification of these compounds?

Presented at the Seventeenth Annual Meeting of the American Diabetes Association in New York City on June 2, 1957.

DR. ROOT: You all have probably reviewed the history of these compounds. I will remind you that some years ago, Loubatières did work in France with an isopropyl thiodiazol and found it lowered blood sugar, but there were side effects which made it undesirable for clinical use. In more recent years, a German group, looking for soluble sulfonamides similar to Gantrisin, noticed reactions in some of their patients which appeared to be hypoglycemic in character. When they investigated further, it turned out that they were producing severe hypoglycemia in some patients. Therefore they started studying the compound in diabetic patients.

This was the compound BZ-55, or carbutamide. Since then Hoechst in Germany developed D-860 which we know as Orinase, and a great deal of work has been done on compounds of this type, studying the structure-activity relationship. There are a number of compounds related to carbutamide and Orinase which lower blood sugar in normal animals and in diabetic patients. About all that can be said at the moment is that the sulfonylurea part of the molecule seems to be necessary for any great degree of activity. It is possible to modify the structure at both ends of the molecule and still maintain some degree of activity.

DR. COLWELL: Dr. Levine, isn't it strange that sulfonamides should lower blood sugar? How do they exert this action?

DR. LEVINE: It is impossible to give all the available evidence in one or two minutes. The β cells of the islets of Langerhans are sensitive to the action of the sulfonylureas. There is some evidence that as a result of such action a small amount of insulin is ejected from the β cells under the influence of sulfonylureas. The presence of the pancreas appears to be necessary for the

drugs to show effects. The presence of the liver does not seem to be essential. There is some evidence that in the absence of the pancreas, but in the presence of exogenous insulin, some potentiating effect is present. The mechanism of this potentiation is unknown.

One thing that seems clear, however, is what the sulfonylureas do not do. They are not substitutes for insulin and do not operate in vitro on insulin-sensitive tissues by increasing their glucose entry or utilization. And they do not operate via the known endocrine antagonists of insulin. Sulfonylureas are still active in the absence of the hypophysis, the adrenals, the thyroid and the gonads. Either endogenous insulin or exogenous insulin at certain dosage levels is necessary for the action of the sulfonylureas. This is about as far as one can go at the moment.

DR. COLWELL: Thank you, Dr. Levine. You are always very lucid on this subject. Dr. Duncan, how are the sulfonylureas used in practice? Which patients are responsive? In which diabetics should they be used?

DR. DUNCAN: In general one can say that sulfonylurea compounds should not be used in patients whose diabetes can be controlled without drug therapy and yet maintain adequate nutrition. With rare exceptions these compounds are not effective in the younger age group, but they correct hyperglycemia and glycosuria in most patients who have developed diabetes in adult life and who are over forty years of age.

I fear there will be a temptation to give these drugs to diabetic patients who are obese rather than reduce weight by food restriction. This would encourage the maintenance of obesity, which is known to contribute to the development of degenerative changes.

DR. COLWELL: Dr. Dolger, do you want to comment on this?

DR. DOLGER: There are two problems here. One is that the longer we use Orinase, the more difficult it is to anticipate which patients will respond clinically. Some with high insulin dosages do very well, while others with mild diabetes do not respond despite a maximum dose.

The second problem involves the selection of patients by the physician. The best candidate is the relatively mild diabetic who has insignificant glycosuria but who is unable to maintain weight on a limited diet. In order to raise the diet the physician will prescribe Orinase. When the physician gains experience with the drug he will expand its use to include persons taking insulin.

DR. DUNCAN: For clarification, a patient may take more than 100 units of insulin daily, but not necessarily have severe diabetes. An obese person who has mild diabetes may tolerate this amount of insulin. This does not mean that he should be given insulin. He should have his weight reduced instead, and not use either insulin or Orinase.

DR. COLWELL: To start the specific questioning period I suggest that we establish an objective. Suppose we first discuss what are the features which make a drug ideal for use by mouth in maintenance therapy of diabetes.

DR. LEVINE: I think the ideal agent would be one which is orally effective, does not irritate the gastrointestinal tract, does not have any toxic effects, and has the established actions of insulin.

DR. DUNCAN: It might be well to emphasize that insulin is dependable in patients with acute infections and ketosis, while the oral preparations are not.

DR. ROOT: Fundamentally the ideal is something with a controlled release factor that would be activated by rising blood sugar and deactivated by falling blood sugar.

DR. DOLGER: I should also like the ideal agent to prevent degenerative changes.

DR. COLWELL: It should be useful in all diabetics, also. Dr. Dolger, how do you think tolbutamide qualifies as an ideal substance?

DR. DOLGER: The answer is simple: it is not the ideal.

DR. COLWELL: Dr. Root, do you want to give us an idea of dosage? Let us take the animal evidence first. What is the effective range of dosage in animals, and how does this compare with human dosages?

DR. ROOT: The first thing to remember is that, in general, effective dosages of pharmacological agents in laboratory animals are usually larger than those used clinically. This is not true for every drug, but it certainly is true of many. Although there is some difference between carbutamide and tolbutamide, the dosage necessary to lower blood sugar in the normal animal is considerably higher than in man. If converted to man, the minimal dose which is effective in rabbits and rats would be equivalent to about 3 gm. in a 70-kg. man. Dog dosages are slightly lower, perhaps equivalent to 1.5 to 2 gm. for a 70-kg. man. Three times this dose in the dog and other animals can be used without toxic effects. These doses produce about the same blood levels as those considered desirable in patients.

There are some species differences in mechanism of action and rate of metabolism of the drug. The monkey tolerates these compounds very well. One can give as much as 150 mg. per kg. per day to a monkey without producing toxic reactions. This would be equivalent to

a dose of approximately 10 to 11 gm. for a 70-kg. man. There have been few signs of toxicity in normal animals until the dose and the blood level are much higher than any used clinically. In such situations the animals develop anorexia and usually die after several days. No tissue changes can be detected in most of the animals.

DR. COLWELL: Is there any distinction between carbutamide and tolbutamide in these respects?

DR. ROOT: There is no difference except for a slight difference in effective dosage.

DR. COLWELL: Dr. Dolger, will you discuss dosage in clinical treatment?

DR. DOLGER: There are three types of patients. In the mild diabetic on a diet a small dose of Orinase (0.5 to 1.0 gm. daily) may be effective. The patient with untreated diabetes who is desirous of not using insulin can be given a dose of 3 gm. This is reduced as results warrant. The patient taking insulin should begin with a 3-gm. dose daily. Insulin is reduced accordingly, depending on the dose. If it is 10 units, we would stop it. Anything over about 10 units is cut down in steps of 10 to 20 per cent daily. A patient taking 100 units of insulin would drop 10 units per day, or 20 units every two days, as a trial. At the first sign of failure, best indicated as increasing glycosuria, Orinase should be abandoned and the original insulin dose resumed.

DR. COLWELL: We will come back to the question of transition from insulin to the drug a little later on. Coming now to toxic reactions and side effects, I should like to be quite practical. Dr. Root, why was carbutamide withdrawn from clinical testing?

DR. ROOT: In clinical trials with carbutamide in about 10,000 cases, the incidence of side effects appeared to be at least 5 per cent. This would not be important if the side effects had been mild, but they ranged from mild skin rashes to agranulocytosis, exfoliative dermatitis, one case of interstitial myocarditis, and two cases of apparent liver damage.

DR. COLWELL: I should like to supplement this with a little information on tolbutamide (Orinase), which Dr. O'Donovan of The Upjohn Company was kind enough to give me a short time ago. Apparently tolbutamide is less toxic than carbutamide. It causes fewer side reactions and they are less serious. This is generally attributed to the fact that the benzene ring has a CH_3 group in the initial position instead of an NH_2 group.

O'Donovan has data on about 5,000 cases collected as of April 1957, from physicians making clinical trials of tolbutamide. The longest any patient had used the drug was about eighteen months. About 170 of these 5,000 cases had had reactions of some kind, an incidence of about 3 per cent. The largest number, about 1 per cent of the total, showed nausea, vomiting and anorexia. Nausea and vomiting due to diabetic acidosis which followed withdrawal of insulin were included. This may well be the chief danger, now that the drug is going into widespread use. Some sixty patients had mild skin reactions. There was no exfoliative dermatitis. Thirty-three had miscellaneous reactions such as headache, weakness and paresthesia. There were eleven leukopenias, with white counts of 1,800 to 3,000 but without any severe depression of granulocytes. A good many of the milder reactions subsided even though the drug was continued.

There have been two good studies of hepatic function, one by Dr. Marble in Boston and the other by Dr. Sherry in St. Louis. Both agree that there were virtually no functional signs of hepatic damage within the limits of the experimental conditions. When there were changes, they most often appeared as BSP retention and increased alkaline phosphatase. They were not striking, were inconstant, and very often temporary.

Dr. Duncan, it was reported yesterday that degranulation of the beta cells occurs, at least in experimental animals. What about this? What is the possibility of islet-cell exhaustion?

DR. DUNCAN: In Allen's work, and later that of Lukens in which animals were made diabetic by removing a large portion of the pancreas and then overfed, there were opportunities to observe the decrease in islet cell function precipitated by overfeeding. This would be a good type of experimental study with the sulfonylurea compounds. We have seen a few patients in whom glycosuria actually increased following the use of these drugs. Two belong to the group which probably produce little or no insulin of their own, namely the juvenile type. Hence it is unlikely that the increased glycosuria was due to exhaustion of the islets of Langerhans.

What has concerned me more is that an increasing number of patients on oral therapy have had to return to insulin, despite an apparently favorable effect from the drug, after four to ten months. One wonders if those patients could not tolerate stimulation of the islets. One can picture reserve islet function that may stand stimulation and produce sufficient insulin to do the job without harm. In other cases continuous stimulation might cause islet cell exhaustion. Of course, clinic patients might do well with oral therapy, and then be careless with the diet. Yet, I have followed several private patients in whom I have complete confidence. In each of three patients we had proved the need for insulin to control the diabetes at a satisfactory state of nutrition. Tolbutamide was shown to be equally effective. Each of

these patients now has had to resume insulin to keep the diabetes under control.

DR. COLWELL: How about the concurrent use of the sulfonylureas with other sulfonamides, with barbiturates or with alcohol?

DR. DOLGER: Everything is compatible with Orinase except alcohol. Following its ingestion some of our patients have developed a cerise color of the skin. The administration at the same time of other sulfonamides, antibiotics, tranquilizers, or barbiturates has not interfered with the effectiveness of Orinase or vice versa.

DR. DUNCAN: I would be interested to know if any of Dr. Dolger's patients have had to return to insulin therapy after having had initially a satisfactory response to oral therapy?

DR. DOLGER: Between 5 and 10 per cent of our group using Orinase showed signs of relapse after four or six months. Mounting glycosuria and hyperglycemia justified discontinuance of Orinase and return to insulin. In these instances the subsequent insulin dose was no higher than that used originally. The explanation for such a relapse eludes us. In only a few people does it seem to be related to an increased diet or gain in weight. In many diabetic patients there is a rising need for insulin, of course, with passage of time.

DR. COLWELL: Do we know anything about the use of sulfonamides in renal disease? How about their use in pregnancy?

DR. LEVINE: You have touched upon two subjects about which there is very meager information. In the Kimmelstiel-Wilson syndrome the drug might accumulate in the blood due to renal insufficiency and lead to side reactions not manifested otherwise. Pregnancy usually occurs in diabetes of juvenile onset which has been treated with insulin until the fertile period. In those instances one would not expect responsiveness to the drug.

DR. COLWELL: It should be pointed out that in contrast to carbutamide it is very difficult to follow blood levels with Orinase. The methods are difficult to establish in the ordinary laboratory.

How about the effectiveness of the sulfonamide compounds in experimental diabetes?

DR. ROOT: Much of this was covered by Dr. Ricketts and in the discussion of his paper this afternoon. The sulfonamides are not effective in the totally depancreatized animal. That goes for the dog and also for the monkey. If alloxan diabetes is severe the drugs are not effective in a variety of animals. Our experience with the use of a sulfonylurea plus suboptimal insulin dosage has given results similar to those reported by Dr. Ricketts in the dog. The same thing happens in the alloxan diabetic dog, and there is some indication that it occurs, also, in the depancreatized monkey.

The type of toxic reaction described by Dr. Ricketts has been found in Dr. Best's laboratory, by Dr. Schambye in Denmark, and by our group. It occurs with carbutamide, with tolbutamide and with several other similar compounds. It seems to be inherent in all of the compounds that we have looked at so far. I didn't get a chance to reply earlier to Dr. Williams' comment that it may be due to a lack of exocrine secretion from the pancreas. The same type of toxic reaction occurs in the alloxan diabetic dog as in the depancreatized dog.

The damage seems to be in the liver. There is a gradual increase of serum alkaline phosphatase and glutamic-oxalacetic transaminase, together with a gradual decrease in serum proteins, especially in the albumin fraction. Although we have not seen it very early, in the later stages there is prolongation of prothrombin time, with capillary bleeding in the intestinal tract.

DR. COLWELL: How about the clinical counterpart of experimental diabetes in which the drugs are ineffective, Dr. Levine?

DR. LEVINE: They are ineffective in the juvenile cases. The major reason for believing that the main activity is exerted on the beta cell is the almost perfect correlation of the degree of effectiveness with the age of onset of diabetes. This may be correlated with Wrenshall's studies in Best's laboratories, which show a similar correlation between the insulin content of the pancreas at death and the age at onset of diabetes. The sulfonylureas are not active in the depancreatized human, either with or without insulin. There is some potentiation of insulin in dogs. This may be a question of species difference. I hate to invoke species difference, because that is always the easy way out.

DR. COLWELL: There has been a good deal of comment about screening tests for the selection of these drugs. Dr. Duncan, is there a good routine method of selection?

DR. DUNCAN: Three groups can be ruled out at once as not being suitable candidates for such therapy. First, patients with acute complications, infections, fever ketosis, and many surgical conditions; second, those who do not need insulin under ordinary circumstances to control the diabetes; and third, juvenile diabetics. More than 70 per cent of uncomplicated diabetes occurs in patients who are overweight. That reduces greatly the number of patients for whom this drug might be indicated, if you agree that diabetes in the obese should be controlled by reducing weight.

We went through three different phases in screening our patients. First, we wanted to find out how quickly and to what degree the drug acted, and the duration of its blood-sugar lowering effect. We divided the diet into twelve equal liquid formula feedings and gave one of these every two hours around the clock. This is not to be considered a form of treatment. After having reduced the insulin sufficiently to allow hyperglycemia and glycosuria, we were then able to observe the rate and degree to which the drug reduced the blood sugar and the duration of its effect. The chief handicap of this method was that it entailed several days.

In the second method the insulin was also reduced to provoke glycosuria and hyperglycemia—a reduction of 10 or 15 units was usually adequate. A blood sugar determination was then done before each meal and after bedtime for one control day. These tests were repeated the following day when tolbutamide was given. By contrasting the two curves an approximate effect of the drug could be estimated.

Recently we have used a more satisfactory test for identification of patients who do not respond favorably to tolbutamide. Such patients need insulin, of course. The dose is reduced by one-half or even omitted if the patient comes into the hospital. (That is desirable in questionable cases.) Following the sharp reduction or omission of insulin and the simultaneous administration of tolbutamide (1 gm. with each meal on the day of the test) each successive urine specimen is tested for sugar and ketones. If a severe grade of glycosuria appears and does not subside it is unlikely that a favorable response will be seen with prolonged therapy. If acetone appears in the urine in increasing amounts within twelve hours, it is a danger sign. Such a patient will not be benefited by tolbutamide and must return without delay to his former insulin therapy. If ketones do not appear, it does not necessarily mean that the patient will be benefited.

The potential danger of abrupt withdrawal of insulin deserves emphasis. Patients who have a real need for large amounts of insulin will be exposed to less risk if they are admitted to the hospital for initial precautionary testing.

DR. COLWELL: Does anyone want to add anything concerning the transition from insulin to sulfonylurea?

DR. DOLGER: I am against hospitalizing patients for initiation of Orinase therapy, because when it becomes available to everybody there will not be enough hospital beds. Secondly, it entails unnecessary expense, besides the trauma of hospitalization. Yet, an uncooperative patient is one that we can not trust. We have had three serious episodes of ketosis in patients who deliberately misled us regarding their symptomatology when we were reducing the insulin to a lower level. These are patients whose eagerness to go off insulin is such that they will give false statements as to their symptoms, and deny the existence of glycosuria. The key to success is close cooperation with the patient when the transition is made, since there is no way of judging which patient may go into ketosis rather rapidly.

DR. COLWELL: Dr. Levine, except for the brief transitional period in which the transfer is made, is there any point in the combined use of insulin with Orinase?

DR. LEVINE: No. At first there was the thought that one might smooth out the labile diabetic by a combination of a smaller dose of insulin with Orinase. This has not been confirmed.

Since the major advantage of the drug is its oral effectiveness, I can see no point in giving one hypodermic injection and one oral pill instead of giving one hypodermic injection alone.

DR. COLWELL: We have two related questions. Do high doses cause hypoglycemia as with insulin? Is there any relationship between increasing dosage and response?

DR. DUNCAN: Yes. I think that the best response is obtained with a relatively small dose. We do not like to give more than 2 gm. daily to start with, and not more than 1 gm. daily in continued treatment. It has been shown that if the dosage is increased, as we might increase insulin to a very large dose, it may actually intensify the hyperglycemia rather than alleviate it.

DR. COLWELL: Will Orinase make labile diabetes more stable?

DR. LEVINE: I would say no. What do you say?

DR. DUNCAN: No, but an open mind should be kept on this aspect.

DR. DOLGER. No.

DR. LEVINE: We agree on one thing.

DR. ROOT: I am sorry, but my patients are all animals.

DR. COLWELL: When does a single dose of Orinase reach its maximum effectiveness during the day? When is the maximum action in the average diabetic patient as distinct from the normal?

DR. LEVINE: Probably between four and six hours; at most eight. Therefore, it might be most rational to divide the daily dose into two equal parts.

DR. COLWELL: Is there general agreement on this point?

DR. DOLGER: I could not demonstrate the difference between 3 gm. once a day and the same amount given in divided doses. Most of our patients take it as a single dose in the morning.

DR. DUNCAN: By dividing the day's nourishment into twelve equal amounts given at two-hour intervals, the greatest effect of a single dose of tolbutamide can be shown to occur between four and six hours. We have abandoned single in favor of divided doses for this reason.

DR. COLWELL: Suppose I am a doctor in practice who does not know much about this subject. Should I prescribe the drug for any and all diabetics? Only for those who use insulin? How much insulin? Only for those who do not?

DR. DUNCAN: Patients for whom oral therapy should be prescribed are those who, under usual circumstances, would require insulin and yet respond favorably to the oral medication. To this degree the drug is a substitute for insulin.

DR. DOLGER: I can think of many specific instances where Orinase can be prescribed. There is no doubt that for handicapped diabetic patients, those visually impaired, those whose problems of intelligence preclude taking insulin properly, in the socio-economic situation that exists in a fairly significant part of our population—if such diabetics can be taken off insulin and put on Orinase safely a great boon would be achieved. When it works, it is of great value to patients suffering from recurrent hypoglycemia due to insulin. Where getting meals or snacks on time is difficult, Orinase is more than a convenience; it improves the whole life situation.

DR. COLWELL: What contraindications are there to the use of the drug, Dr. Levine?

DR. LEVINE: The first is juvenile diabetes; the second is diabetes following pancreatectomy or pancreatic disease; the third is diabetes during pregnancy, because we do not know anything about it; the fourth might be renal complications; the fifth are those severe diabetics who may have been adults at onset of the disease, but who behave like juveniles. In other words, all juveniles are not young.

DR. DUNCAN: I would add to this rather complete list those patients having acute infections and ketosis.

DR. COLWELL: There has been some confusion about albuminuria in people taking Orinase. Dr. Dolger, do you want to say something about that?

DR. DOLGER: The administration of Orinase produces a flocculent precipitate when ordinary albumin tests of the urine are made. This precipitate is due to the excretion of a carboxylated product of Orinase. This will make it appear that the patient has albuminuria. The brochure distributed by the manufacturer describes the technic for distinguishing between pseudo and actual albuminuria. A simpler and accurate method for detecting albuminuria in the presence of a confusing precipitate of Orinase excretory product is the Albutest tablet of the Ames Company.

DR. COLWELL: We might use the last five minutes for each member of the panel either to discuss the papers on "DBI" which were given this afternoon, or else to answer some of the questions which are before them. There are a few which cover items that we have not discussed.

DR. ROOT: I am not competent to say anything about the "DBI" work. We probably have heard today all that has been done with it.

I would like to point out one more thing about the sulfonylureas. One sees it in animals as well as in patients. The drug's major effect seems to be on the fasting blood sugar. The effect on the postprandial blood sugar is less than one sees with insulin.

DR. LEVINE: I have a question here which is interesting. "Is there any indication that a classification of patients responsive to the sulfonylureas might serve to divide the syndrome of diabetes mellitus into further component entities?" In a way, yes. Indication can be found in the work on sulfonylureas, on the inhibitors of insulin action, and on the carriers of insulin in the plasma. Insulin assays indicate that the syndrome of diabetes mellitus, which we divide roughly into juvenile and adult onset varieties, might be classified instead into groups of individuals who do not manufacture insulin; those who manufacture insulin but do not easily release it in response to the normal stimulus; those who release it but have a substance in the plasma that inhibits its action; those who release it and have an inhibitor only during ketosis; and perhaps other varieties. We are approaching an era of classification on biochemical grounds. If the sulfonylureas do nothing else they have stirred up everybody concerned with diabetes.

DR. COLWELL: No one has pointed out yet today that the mechanism by which these substances act may be of even more importance than the drugs themselves.

DR. DUNCAN: I have several questioners to answer. The first asks "How do you correlate the following: Following glucose, given in a glucose tolerance test, there is a rise in the blood sugar. On Orinase therapy there is a fall following one-twelfth of the diet?"

In the glucose tolerance test, there is rapid absorption of a large amount of glucose, whereas the small feeding is absorbed more slowly and more uniformly. Furthermore, no blood-sugar lowering agent is given prior to the glucose tolerance test.

"Is not allergy to insulin an indication for Orinase?"

It is not particularly an indication for Orinase, but

rather a reason to find out to which insulin the patient is allergic, and whether he is allergic to the added protein or to the insulin molecule itself. If he is allergic to regular insulin, by all means desensitize him so that, if acute complications occur, he will not be deprived of its use. To use Orinase in place of insulin and do nothing more about insulin allergy would mean avoidable exposure to future difficulties.

"In a patient who has a blood sugar relapse after several months of therapy when do you discontinue Orinase?"

Discontinue it as soon as you decide Orinase is having no effect.

"How do you persuade the patient it is necessary to stop Orinase?"

By pointing out that his regimen is not adequate to control the diabetes.

"In resuming insulin therapy after failure with an oral agent, do you encounter sensitization to insulin?"

We have not had this experience. Such cases must be exceedingly rare.

"Are there other difficulties to be anticipated in the resumption of insulin?"

There are no difficulties. If oral therapy is inadequate, the insulin taken previously by the patient can be resumed promptly.

DR. DOLGER: In the light of this morning's conference and this afternoon's presentation, is it not logical to assume that a drug whose disadvantage is supposed to be that it works only via endogenous insulin may be superior to exogenous insulin?

DR. COLWELL: We must close now. On behalf of Dr. Marble, the Chairman of the Program Committee, and of the audience, I would like to thank the panelists for their interesting comments, and on behalf of the panel, to thank the audience for their attention and interest.

A Thirty-fifth Anniversary of Insulin Therapy and a Sixty-fifth Wedding Anniversary

H. I. Burtness, M.D., Santa Barbara, and E. F. Cain, M.D., Anaheim, California

The January 1956 issue of DIABETES commemorated the thirty-fifth anniversary of the discovery of insulin. Appropriate tribute was paid to the discoverers of insulin in Toronto, Sir Frederick G. Banting and Prof. Charles H. Best, and to Leonard Thompson, the first patient to receive insulin in Canada. We would like to supplement that report this year by reporting the case of Charles E. Cowan of Anaheim, California, a patient of the late Dr. William D. Sansum. According to our information, he is the first patient in the United States to receive insulin made here, and in May 1957 he celebrated his thirty-fifth year of insulin therapy. Mr. and Mrs. Cowan celebrated their sixty-fifth wedding anniversary on April 12, 1957. Such a celebration was an event in itself, but of greater significance was the fact that Mr. Cowan had lived to make it possible.

Mr. Cowan came to the Potter Metabolic Clinic in Santa Barbara on Nov. 8, 1920, the same day that Dr. Sansum arrived from Chicago to be director of the clinic. A brief history of this clinic may be of interest. It was established in 1919 by Dr. Nathaniel Bowditch Potter of New York City for the purpose of studying metabolic disorders, particularly diabetes, from the research and clinical viewpoints. Dr. Potter was a diabetic patient himself. Because of failing health, he had come to Santa Barbara and was unable to return to New York. Contributions, including funds for the project from the Carnegie Corporation, were transferred to Santa Barbara. With the financial assistance of friends here the Potter Metabolic wing of the Cottage Hospital was built. Dr. Potter died in July 1919, before the building was completed. In 1928 the Potter Metabolic Clinic be-

came a part of the Cottage Hospital. However, between 1920 and 1928 it was the scene of historic events in the field of research and treatment of diabetes, a fitting memorial to Dr. Potter.

This clinic had the honor of being the first place in the United States where insulin was manufactured and successfully used. Experimental work on diabetes had been started along other lines in the clinic laboratories when insulin was discovered in Toronto in 1921. Because the Canadian reports published in February and March of 1922 offered greater promise of success, and because the clinic was fully equipped for the study of diabetes, work was started immediately on insulin extraction. In April, Dr. Sansum and his chief chemist, Dr. Norman R. Blatherwick, had succeeded in making a small amount of insulin. By May, they had sufficient insulin to start work on nine patients of whom Mr. Cowan was the first. A report of the work was sent to Prof. J. J. R. Macleod in Toronto in June, and exchange of information regarding insulin continued during the experimental years. Important contributions were made from this clinic, many of which were adopted by pharmaceutical houses later. By 1925 the manufacture of insulin was discontinued here, as an ample supply was available from Eli Lilly and Company. This company had been invited in May 1922 to assist Dr. Banting in the commercial manufacture of insulin and by August had produced a product that was very stable. The company had the privilege of making available the first commercial insulin in the world. Some of the methods of extraction and purification of insulin worked out in the laboratories of the Potter Metabolic Clinic were adopted by this pharmaceutical house, and the method devised in this laboratory of estimating the strength of the insulin units is still in use. The report of the first 100 patients treated with insulin manufactured here was published in 1923.[1]

Mr. Cowan had developed diabetes after a severe case of influenza in 1919, one and one-half years prior to coming to the clinic and three years before the discovery of insulin. He was fifty-one years of age, five feet eight inches in height, with a large frame and weighed 112 pounds. He was admitted with a severe case of diabetic acidosis and there was little hope for his life. However, he recovered from this acute condition and returned to the clinic in 1921 to remain for a number of years as a "human guinea pig." He received his first insulin in May 1922. At that time he weighed 95.5 pounds and had been existing on a diet of 844 calories, containing 24 gm. of carbohydrate, 34 gm. of protein, and 68 gm. of fat. The strength of the first insulin is not recorded but it must have been quite weak as he was given 3 cc. one hour before each meal at first and later 2 cc. When the first supply was exhausted the amount was reduced to 1 cc. before each meal, as it was stated that the next insulin obtained was twice as strong as the first. By July 15, 1923, he weighed 125 pounds on a diet of 2,993 calories, containing 91 gm. of carbohydrate, 79 gm. of protein, and 257 gm. of fat. On April 16, 1924, his weight was 153 pounds on a diet of 2,417 calories, containing 91 gm. of carbohydrate, 79 gm. of protein, and 193 gm. of fat.

During the period before the discovery of insulin, various forms of treatment and diet had been used on Mr. Cowan, ranging from yeast and bran to very high fat diets. In 1925, Dr. Sansum started experimental work with higher carbohydrate diets,[2,3] which made it possible to feed diabetic patients adequate diets for the first time in this country. As soon as possible Mr. Cowan was placed on a higher carbohydrate diet consisting of 2,334 calories, containing 235 gm. of carbohydrate, 83 gm. of protein, and 118 gm. of fat. He has continued with this type of diet to the present time. His weight has been maintained at approximately 148 pounds for several years.

Mr. Cowan continues to test his urine twice daily. He has very few insulin reactions and they are mild. His insulin dosage consists of 40 units of U80 NPH insulin daily before breakfast and occasionally 5 units of regular insulin when the need arises. This dosage has been essentially the same for several years.

He still retains useful vision as he is able to read ordinary print without the aid of glasses. There are lenticular opacities present, making examination of the eyegrounds unsatisfactory.

The only complaint is pain in his feet, especially when walking. He has had pernicious anemia since 1938, controlled by liver extract, discovered by Dr. G. R. Minot and Dr. W. P. Murphy. (Dr. Minot was a diabetic whose life was saved by insulin.) A recent blood count showed 16.6 gm. of hemoglobin, 4,500,000 red blood cells, and 8,300 white blood cells, of which 60 per cent were polymorphonuclear leukocytes and 40 per cent lymphocytes. The platelets were normal.

His general physical condition shows no great abnormalities except that the tendon reflexes of the knees and ankles are not obtainable. The pulsations in the dorsopedal and posterior tibial arteries are not palpable. There is no edema of the lower extremities. The chest is clear. The heart rate is 72 per minute, when he is resting in a chair, rhythm is normal and there are no murmurs. His blood pressure is 142/80.

Mr. Cowan is now eighty-nine and his wife eighty-four years of age. He is mentally alert and carries on a conversation concerning current events, as well as reminiscences regarding his long and interesting life. He has outlived his doctor and his allotted time of three score years and ten and credits his longevity to both insulin and an adequate diet. Mr. Cowan's life should be an inspiration to all diabetic patients and a great satisfaction to the people still living who worked so diligently on insulin in the early days. He and his wife give daily thanks to those who saved his life and made it possible for him to live a normal, happy life.

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EDITORIALS

IS THE METABOLISM OF PERIPHERAL TISSUES AFFECTED BY THE ARYLSULFONYLUREAS?

In this issue of the Journal a paper by Ashmore, Cahill, Earle and Zottu[1] reports experiments in which the actions of insulin and tolbutamide in one and the same experimental preparation are compared. This paper prompts a review of the experimental evidence reported in the recent literature on the effects of the arylsulfonylureas in experimental and clinical diabetes. The conclusion seems to be warranted that the main action of these drugs is pancreatropic, bringing about a release of insulin from the β-cells. This conclusion is based on the unequivocal evidence that in the absence of the pancreas and in instances of complete alloxanization, as well as in the Houssay animal, no hypoglycemic action of the drug is demonstrable. The insulin released from the pancreas by the action of the drug, through its effect upon the metabolism of the liver, brings about a diminished output of hepatic glucose resulting in hypoglycemia. However, it is impossible to rule out that the sulfonylureas, aside from the action of the released insulin itself, may have some direct action upon hepatic mechanisms through which the release of sugar is diminished. For example, evidence has been presented indicating that certain hepatic systems, particularly glucose-6-phosphatase, may be inhibited, thus increasing hepatic glucose retention.

One of the main obstacles in accepting this thesis as an exclusive hypothesis for the action of the sulfonylureas is the difficulty in excluding unequivocally the peripheral action of the drug. Endogenous insulin released in response to the pancreatropic action of the drug should be expected to reach the periphery and exert its customary effect there. However, experiments from many laboratories have failed to demonstrate an unquestionable insulin-like action in the periphery following the administration of these hypoglycemic agents. Indeed, the significance of the paper by Ashmore et al. lies in the demonstration that a direct comparison of the respective actions of tolbutamide and insulin upon peripheral tissues (muscle and fat) shows that they are quite different. In detail, the experiments of Ashmore et al. show that tolbutamide action brings about a cessation of hepatic glucose output. In contrast, insulin increases it. After isotopic glucose administration the hypoglycemia following tolbutamide does not increase the decay in blood glucose specific activity greater than that of a saline control, but following insulin administration it is markedly accelerated. Furthermore, when identical blood glucose changes are produced by tolbutamide and insulin the former has no significant effect upon the incorporation of C^{14} from isotopic glucose in either peripheral glycogen or fatty acids, in sharp contrast to the significant effects which insulin produces in these two metabolic reactions. From the summation of their evidence Ashmore et al. conclude that the action of tolbutamide appears to be limited to the pancreas and the liver.

In general, these conclusions are in conformity with the experimental data published by others, but there is sufficient authoritative data leading to opposite conclu-

sions so as to render dogmatism dangerous. A brief summary of negative evidence for peripheral action of the sulfonylureas is outlined. Wick[2] has been unable to demonstrate an acceleration of CO_2 production in animals injected with isotopic glucose following administration of hypoglycemic agents. In thirteen normal and six diabetic patients Elrick and Purnell[3] were unable to show any increase of the A-V/A values following the administration of tolbutamide. In contrast, there was a significant increase in this measurement following insulin administration. Furthermore, following tolbutamide they were unable to demonstrate any enhancement of insulin action on peripheral utilization of glucose. They concluded cautiously that "it is conceivable that the action of exogenous insulin differs somewhat from that of endogenous hormone by reason of its preparation and/or route of administration." Goetz, Senn and Senn[4] reported changes in capillary blood sugar differences, and in venous potassium and inorganic phosphorus following injections of tolbutamide quite similar to the effects observed after insulin. However, in a later paper[5] they were unable to demonstrate any change in nitrogen balance or respiratory quotient in two diabetic patients: "These results do not accord with the acute experiments reported earlier which imply an effect of tolbutamide on the peripheral utilization of sugar." In the light of this they speculate upon the possibility of an acute effect of tolbutamide resulting in a blood sugar fall following insulin release whereas the chronic effect is due to the action of the tolbutamide upon the liver. The release of insulin is too small to be detected by the methods used. Frawley, Segal, Camus and Foley[6] were unable to show that tolbutamide accelerated the rate of disappearance of xylose and arabinose from the blood in normal male and female subjects eighteen to twenty-one years old. In contrast, insulin significantly accelerated the rate of fall of blood concentration of these pentoses: "Tolbutamide is devoid of an insulin-like action on pentose although a hypoglycemic (decreased glucose concentration) effect was obtained. These observations point to a major difference in the mechanism of the action of insulin and tolbutamide." The main action of the sulfonylureas appears to be chiefly upon the glucose metabolism of the liver. They speculate upon the possibility that the liver in some unknown manner becomes more responsive to endogenous insulin when the sulfonylureas are administered. This enhanced effect for unknown reasons is not manifest in the periphery. Renold, Martin, Boshell and Thorn[7] studied the possibility of peripheral action of tolbutamide using the following indices: intravenous glucose tolerance, serum phosphate levels and blood pyruvate levels after intravenous glucose, respiratory quotient and nitrogen balance. They were unable to find that any of these indices were changed by the administration of tolbutamide. Some effects were observed upon the levels of blood ketones and of unesterified fatty acids which resemble the effects observed after insulin. In addition, they measured plasma insulin-like activity using the method of Vallance-Owen and in no instance were they able to find any increase of this following tolbutamide administration. They conclude that "increased peripheral glucose utilization is not a major component of tolbutamide action." Recant[8] published evidence which supports the hypothesis that the primary action of the sulfonylureas is upon the metabolism of the liver. She studied the incorporation of isotopic amino acids into liver proteins, reactions which are dependent upon adequate utilization of carbohydrate by that organ. Liver slices taken from Sprague-Dawley rats were equilibrated in vitro, and the incorporation of C^{14}-glycin into liver protein was measured. There was a significant increase of this in the tolbutamide-treated rats. In addition, she observed in vitro that these liver slices showed a significant decrease in hepatic glucose release and hepatic pyruvate formation when compared to slices from untreated rats. In contrast, neither the glucose uptake nor the pyruvate formation by the isolated rat diaphragm was altered following tolbutamide administration. Peripheral glucose utilization as determined from the A-V glucose difference in the human subject was not changed by treatment with the drug. Her conclusion was that the action of tolbutamide is essentially upon the liver alone and simulates the effects of insulin. Dulin and Johnston[9] compared the action of insulin and tolbutamide upon liver and muscle glycogen. They report that tolbutamide increases liver glycogen but does not alter muscle glycogen in fasted intact animals, whereas insulin increases muscle glycogen with no significant change in liver glycogen. However, their general conclusion is that tolbutamide acts to stimulate the secretion of insulin by the β-cells of the pancreas. To explain the absence of peripheral insulin effects in the face of this increased secretion of endogenous insulin, they point to the fact that a constant intravenous infusion of small doses of insulin can mimic tolbutamide action by causing a significant depression of blood sugar but no change in muscle glycogen. In other words, the tolbutamide effect is due to a prolonged action causing a steady release of small amounts of insulin. Wick, Britton and Grabowski[2] using isotopic tolbutamide measured its volume of distribution after injection. The observed value was 22 per cent of the body weight, ap-

proximately equal to the extracellular compartment. From this evidence they concluded that sulfonylurea can have "no intracellular action on intact muscle cells and the only possible extrahepatic action could be on the cell surface." However, sulfonylureas did not accelerate the rate of transfer of glucose into muscle cells and they concluded that it does not have direct action on this tissue. Renold, Winegrad and Froesch[10] studied the action of the arylsulfonylureas on peripheral glucose utilization in six patients with mild diabetes and in one normal subject. After glucose loading the rate of disappearance of glucose from the blood was no different after arylsulfonylurea administration than in control periods. Measurements of serum inorganic phosphate, blood pyruvate, blood lactate and urinary phosphate also failed to show changes indicative of increased peripheral glucose utilization following administration of these hypoglycemic agents. Purnell, Arai, Pratt, Ahlad and Elrick[11] employed essentially the same technic as that described in the previous paper. They found no changes in glucose tolerance and phosphorus curves following the administration of tolbutamide. These authors also studied the A-V glucose values in a series of normal and diabetic subjects and were unable to demonstrate any significant effect upon the peripheral utilization of glucose by the drug using this index as a criterion. These authors state that their experiments are not in accord with the hypothesis that the sulfonylureas increase insulin output, since this hypothesis demands increased peripheral glucose utilization. They have no resolution of this anomalous situation. In contrast, Goetz, Gilbertson and Josephson[12] determined the A-V/A ratio in a series of human cases both normal and diabetic. They found that tolbutamide, either alone or following the injection of insulin, significantly increased this ratio. In the absence of the pancreas there was no such effect. The authors state that the increase of the ratio is assumed to mean an increased carbohydrate utilization. However, an increased A-V difference does not necessarily imply a normal fate of the sugar which has disappeared. They make no attempt to reconcile their findings with the contrary findings of previous authors. In general support of the last paper is the study of the utilization of uniformly labeled C^{14}-glucose by rats after tolbutamide by Miller, Krake and Vanderbrook.[13] These authors compared the effect of insulin and tolbutamide on the disposition of labeled blood glucose over a six-hour period. They report that both insulin and tolbutamide increased the rate of incorporation of isotopic glucose into glycogen.

It is not easy to sum this evidence in a few words. The preponderance of evidence supports the pancreatropic hypothesis of the action of arylsulfonylureas coupled with a secondary effect of the released insulin upon the liver. However, such a conclusion demands the formulation of subsidiary hypotheses employing tenuous concepts unsupported by experimental support. In addition, evidence apparently based upon carefully controlled work indicating that these drugs do have peripheral action, must be ignored. As usual in instances of this sort, one must close with the statement that judgment is reserved until further evidence is available.

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DIET AND ATHEROSCLEROSIS

A presentation of the evidence for and against the concept that the fat content of the average present-day North American or northern European diet is a significant factor in atherogenesis has been published by the Nutrition Committee of the American Heart Association.* Entitled "Atherosclerosis and the Fat Content of the Diet," this review finds that existing evidence does not justify recommendations for drastic changes in the amount or type of fats consumed by the general public as a means of preventing atherosclerosis or its sequelae. The report adds, however, that the evidence, incriminating diet as an environmental factor in atherogenesis, is strong enough to warrant early, thorough and uncompromising investigation.

Noting that atherosclerosis in all probability has no single cause, the report states that factors presently implicated include heredity, anatomy of the blood vessel wall, arterial blood pressure, diet, lipid content of the blood, sex and many others. Because animal experiments have shown that atherosclerosis, similar to, but not identical with the human type, can be produced in a variety of animals by dietary variations, the attention of clinicians has tended to focus on the possible dietary factors (as, of course, has that of the lay public).

A basic criticism of this approach, the authors hold, is that most experimental studies have been concerned with concentrations of serum cholesterol, serum lipid or serum lipoprotein, and not with "the real nub of the problem," *atheroma* and *infarction,* whether myocardial or cerebral. Infarction is not generally produced in experimental animals despite the extensive and severe atherosclerosis that has been produced. Compelling evidence is thus lacking from the experimental data that treatment of hypocholesterolemia by dietary means significantly lessens the chances of myocardial infarction. At best, there is an association of statistical value, but this is not an obligatory association, either in small groups or (much less so) in an individual, according to the authors.

Another basic criticism concerns the association frequently drawn between hyperlipemia and dietary fat intake. Although the equation of *atherosclerosis* with *hyperlipemia* is presumably valid, other parameters such as total caloric intake, relative rate of caloric expenditure, true obesity and exercise, are not easily disentangled from the problem of excessive fat intake.

On the other hand, the facts that obesity is a nutritional failure, caused by consuming more calories than expended, that dietary fats are the most concentrated source of calories, providing some 40 to 45 per cent of the daily intake, suggest that many should consume fewer calories.

For most, the authors hold, this will mean eating less fat. Diets providing 25 to 30 per cent of the calories from fat "can still provide palatable meals for our accustomed tastes."

Much attention is given in the report to data derived from population studies. These are often cited to support the view that coronary disease has increased alarmingly in the past generation or two in countries like the United States with high living standards, including high fat consumption.

Concerning prevalence, the authors say: "No one questions the remarkable increase in the *reported* number of cases," but they note that this rise may be due largely to artifacts such as changes made in 1949 in the International List of Causes of Death, acceptance of broader concepts of coronary disease among physicians filling out death certificates, wider use of the electrocardiogram in confirming clinical diagnosis (as well as, of course, the aging of the population).

One of the major problems under discussion, according to the authors, is whether there has been a marked change in the American diet during the past generation or two — a period in which the incidence of coronary atherosclerosis may have increased. Are Americans actually eating more fats than formerly? A clear-cut answer is not available. Studies made among college students during the 1890's indicated that 36 to 44 per cent of calories eaten by these groups came from fats, a ratio similar to that found today. Current Army rations have a fat content almost identical with that of rations served troops on the western outposts during the late 1880's. Moreover, data on the dietary habits of peoples are difficult to analyze. Food availability in a country — such as government estimates of retail food stock — is not the same as food consumption; data on food waste, for example, are not usually reliable. The collection of tremendous amounts of fat from kitchen waste during World War II is cited as an indication of the degree of loss from this one source alone.

Regarding the often-quoted experience of European countries experiencing food and fat shortages during World War II, the authors cite studies that question the conclusion of a concomitant drop in the death rate from arteriosclerotic heart disease. In Britain, for example, cardiovascular mortality declined before fat ra-

* Page, I. H.; Stare, F. J.; Corcoran, A. C.; Pollack, H.; Wilkinson, C. F. Circulation, August 1957; J.A.M.A. Aug. 31, 1957.

tioning was instituted in 1939, but resumed an upward trend in 1943 despite the fact that fat rationing continued and was even intensified in 1947. Similarly in Norway, apparent mortality from cardiovascular disease tended to decrease *before* wartime shortages became acute.

Another question often encountered is whether a change in type of fat eaten may be involved in the possible higher incidence of atherosclerosis. The proportion of animal and vegetable fats in the American diet has remained relatively constant, the authors say, citing U. S. Department of Agriculture tables that show a ratio of 70 per cent animal to 30 per cent vegetable fat available for human consumption both twenty years ago and today. While there has been an increase in consumption of hydrogenated fats, this is not held to have produced a drop in the intake of essential fatty acids. Increased saturation of fats induced by the hydrogenation of shortenings and margarines has been balanced by an increased consumption of other fats and oils, largely unsaturated and with a high content of essential fatty acids, and also by decreases in the use of butter and lard.

The report also calls attention to other common factors in human life which are atherogenic but not related to lipemia. Of these, the most common and best documented is arterial hypertension. Patients with severe hypertensive disease leading to atherosclerotic complications, fatal and nonfatal, have not been found to have a demonstrable association between the incidence of these complications and the levels of serum cholesterol or of the various lipoprotein fractions.

For the general public, the safest guide is "nutritional common sense," or the eating of a balanced, varied diet with caloric intake adjusted to overcome or prevent obesity. The fat content should be sufficient only to meet caloric needs and requirements for the essential fatty acids.

These recommendations do not rule out special dietary regimes for patients or individuals with strong family history of heart disease who are seen by their physicians with some regularity. In such cases, newer concepts of nutrition readily suggest various types of diet therapy which may prove useful to certain patients, and at the same time increase the body of data from which dietary recommendations may be made to the public at large.

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DIABETES IN TURKEY

The incidence of diabetes in Turkey, like its occurrence among the Arizona Indians,[1] is probably the same as it is in Europe and the United States. Joslin[1] gives no data on the occurrence of diabetes in Turkey, and no information appears to be available about the country as a whole. Aksan and Yalcin[2] in 1950 at the Nümune Hospital in Ankara reviewed the reported incidence of diabetes, which has increased steadily since 1928. This increase is due presumably to more diagnostic examinations in new hospitals combined with better national nutrition. Their[2] unpublished figures for the year 1956 show that among 16,323 patients admitted to this City Hospital, diabetes occurred in 0.76 per cent. These figures resemble Joslin's estimate for diabetes in the United States in 1946 (0.7 per cent). As far as large hospitals are concerned, the disease appears with the same frequency in both countries. Life insurance has not developed as it has in North America, and the valuable facts on the incidence and life expectancy of diabetic patients in insured populations is lacking in Turkey. In particular, this means that terminal vascular complications are poorly documented. However, for Turkey as a whole, failure to recognize diabetes is still a problem. This may be illustrated by the experience of the pediatric service at Nümune Hospital. In the last ten years, in a 200-bed children's ward, only ten diabetics had been seen without one single case of coma. When this was called to their attention, the staff agreed that most children with diabetic coma died at home, at least in the villages. Of course, the statistics and the improved diagnosis are continuing problems in every country.

Physicians in Turkey lack the aids which are available for diagnosis and treatment in this country. At the 800-bed University Hospital in Ankara, there was one dietitian with three helpers. She was unable to give any dietary instruction to outpatients. Even at their medical centers the preparation and use of diet lists is minimal. Moreover, no diet lists are prepared and distributed by pharamceutical houses, which have been so helpful in this country. The protein, fat and carbohydrate content of foods is available in Turkish,[2] but the information is seldom arranged as diets with convenient food exchange units. Translation into Turkish and mimeographing of selected diets of the

The Editors of this Journal, believing that wider knowledge of diabetes in all its aspects will be useful to students and practitioners of this subject, invited Dr. Lukens to summarize his impressions of the problems of this disorder as he encountered them on a recent prolonged visit to Turkey.

American Diabetes Association was a first step in the care of patients in Ankara, and it is hoped that this system will be more widely used. Incidentally, when explaining a diet to a Turkish patient, no attempt is made to use quantification by household measures. No uniformity of such household equipment exists in Turkey. However, kitchen scales are used widely by housewives and cooks, and approximate weighing is successful even among ignorant patients.

There are no peripheral vascular clinics, chiropodists, or others to conduct what Joslin has called "beauty parlors for diabetic feet."

Enough insulin seemed to be available at a modest price for those taking a little trouble to obtain it. In use are varied types and concentrations of insulin from many foreign sources. Insulin is not manufactured in Turkey, although there are enough cattle to provide the raw material. Crystalline insulin was prepared in Professor Aras' laboratory in Ankara, and an approximate assay indicated that the insulin content of Turkish cattle is similar to that of American.

Education of both physicians and patients concerning diabetes is generally less advanced than in America. Intelligent diabetic patients are well regulated, mostly from their own efforts, and physicians have an undue fear of insulin because they have not accepted the responsibility of using it. In this connection it is well to remember that Turkey has overcome malaria and trachoma, is rapidly bringing tuberculosis under control, and has made enormous strides in public health. As their most serious problems are solved, their good physicians will turn to the care of chronic disease as we are doing in the United States.

Now and in the future, the first need for progress in diabetes will be a group of physicians to spearhead the campaign of education in this field. The Turkish Diabetes Association, centered in Istanbul, should become truly national in scope. In conclusion, visiting a country where nothing exists that is comparable to the American Diabetes Association is one of the best ways to appreciate our diverse activities at home.

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Friedrich von Müller 1858-1941

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A sincere friendship based on mutual respect and appreciation existed between Friedrich von Müller and Sir William Osler. The personalities of both were entirely different, but their main endeavor in advancing the teaching of medicine was the same. Müller was basically stern by nature while Osler's outgoing, winning personality at once captured the attention of everybody.

The father and grandfather of Dr. Müller were physicians. His mother was from a family of patriarchal merchants in Augsburg. The architectural and monumental beauty of his hometown of Augsburg, the town of the Fuggers and the birthplace of Holbein and Mozart's father, influenced his intellectual development. The impressions a young boy absorbs on his way to school may stimulate his imagination and inventive talents more than several courses of humanities.

Friedrich von Müller started his studies in the famous laboratories of the chemist Adolf von Bayer at the University of Munich. Here he met Emil Fischer, the pioneer of protein and carbohydrate chemistry. A lifelong friendship with E. Fischer resulted from this rather short episode as a chemistry student, as Müller soon changed to a medical curriculum. Carl Voit, Professor of Physiology, attracted at this time many students (e.g., A. Rubner, Graham Lusk). He also influenced the training and ideas of young Müller—"A correct figure is better than interesting but unproven speculations."

Dr. Müller, having finished his medical curriculum in Munich, became Assistant (Resident) in 1882 to Carl Gerhardt, Professor of Medicine at the University of Wurzburg. With his chief, he published during this

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time, in the Bulletin of the Wurzburger Clinic, interesting bedside observations on protein metabolism of cancer patients, emphysema of the mediastinum, and the behavior of the muscles of the larynx during paralysis agitans. In Wurzburg he also became interested in jaundice and especially in the formation of urobilin, subjects which later led him to fundamental experimental observations.

One of Dr. Müller's most important contributions to the teaching of medicine had its beginning in Wurzburg. In 1886 there appeared the first edition of his *Taschenbuch der Medizinisch-klinischen Diagnostik,* published together with O. Seiffert, who wrote the minor part on the examination of the larynx. Characteristic of Müller's loyalty to his friends was the fact that he kept Seiffert's name as co-author despite the fact that Seiffert had nothing to do with the writing of the *Taschenbuch* in later editions. The first edition contained only 100 pages, the fortieth edition almost 600 pages. The *Taschenbuch* was translated into many languages (Ralph Major translated several editions into English). The influence of this precise and skillfully condensed manual on the development of technics for clinical examination was universal before the first World War.

During his whole life, Dr. Müller's task was to teach clinical medicine as a unit. He believed that one should not teach details of specialties to masses of students in the auditorium. The professor should have a comprehensive knowledge of medicine together with a sense of values and a feeling for tradition. Müller indeed exemplified these characteristics to perfection. He always maintained a balance between bedside teaching and laboratory investigations. His ability to draw simultaneously with both hands on the blackboard with dexterity made it possible to picture the anatomical relations in neurological diseases as well as to sketch histological findings of diseased organs during a clinical demonstration of a patient.

Müller next moved with his chief, Professsor Gerhard, as senior assistant to the Charité Hospital of the University of Berlin. In 1889 he was given his first independent position as Chief of the Poliklinik of the University of Bonn, and in 1896 he became Chief of the Poliklinik at the University of Breslau. There his study of neurology was strongly influenced by Wernicke.

The next station of his academic career was the Poliklinik of the University of Marburg. H. H. Meyer, A. Kossel, V. Behring and F. Marchand together with Müller were stimulating members of the Marburg faculty. A few years later, in 1899, he accepted the invitation of the University of Bâle to the chair of Medicine, which he occupied until 1904. The faculty of the University of Munich offered him the position of Chief of the II Med. Clinic and of Professor of Medicine, an office which he kept until he became professor emeritus in 1937.

The scientific activities at the Munich clinic and Müller's lectures soon attracted young physicians from all over the world. The wards of the clinic viewed historically were among the first where diagnostic activities at the bedside were integrated with those of the chemical laboratory. There were, however, no chemical technicians for routine chemistry available. The resident physician had to carry out every determination himself. The residents with the help of a few clinical clerks had to work until late into the night to satisfy the stern chief at the ward rounds the next day. The personal relations of the chief and his co-workers were friendly but always involved a certain restraint. He liked the expression of independent opinion, but one had to find the right words in formulating it.

Beside the bedside work, the residents had to carry on research on basic problems. In Marburg, the chemical test for blood in the feces was worked out (by H. Weber). At this time Dr. Müller found that glucosamin was present in the phlegm of sputum. In Bâle, working with O. Simon, he demonstrated that the fibrinous exudate in the alveoli in pneumonia during the "lysis" was disintegrated by enzymatic fermentation of leucocytes and not reabsorbed before disintegration to amino acids. Erich Meyer and O. Neubauer also demonstrated, while still residents with Dr. Müller in Bâle, the significance of homogentisinic acid in alkaptonuria. O. Neubauer and E. Meyer accompanied Müller to Munich as senior residents. Here leukemia, polycythemia and other blood dyscrasias were investigated by Meyer and W. V. Domarus. Dr. Müller's early studies of urobilinogen and jaundice were continued by Neubauer and the urobilinogen reaction with Ehrlich's reagent was first described. The chemical constitution of the porphyrines was elaborated in Müller's laboratory by Hans Fischer, later given the Nobel prize for the synthesis of porphyrines.

Dr. Müller coined the name "nephrosis" for a group of kidney diseases in his famous paper at a meeting of German scientists in Meran (1905). H. Heinecke as well as P. B. Monakow and O. Neubauer tried to elaborate on Müller's ideas of kidney function with experiments on animals and studies of patients. W. J. Stauffenberg took up Müller's observations on cortical blindness in an extensive study of localization of cerebral function. In fundamental papers, Stauffenberg also described the

diseases of the extrapyramidal tracts. The clinical importance of the so-called "protein minimum" was investigated by Erich Kraus and S. Lanter.

It was my task to investigate purine metabolism in Dr. Müller's laboratory. These studies later led to the isolation and crystallization of mononucleotides (adenylic acid) from yeast nucleic acid (RNA) and from desoxyribonucleotides (DNA). Studies of lipid metabolism, especially of cholesterol, were also started by me and co-workers in Dr. Müller's laboratory. Studies of the effect of intravenous sugar infusion on blood sugar curves of normal and diabetic subjects were published from Müller's laboratories in 1913.

One of the main topics of Dr. Müller's research was the investigation of the basic physical principles of percussion and auscultation and their objective registration by elaborate physical instruments. W. Selling, E. Edens, George Fahr and finally the excellent work of Paul Martini helped the master in this thorny task. Enthusiastically he returned from his second American visit in 1926, telling us that Campbell in Rochester had demonstrated the newest electric resonators and sound filters. Until the end of his life the registration of clinical sound phenomena with electronic amplifiers and filters occupied his mind.

The greatest attraction of Müller's teaching activities was the daily discussion of patients before the students in the large auditorium of the hospital. Each morning at 9 a.m. the master entered the auditorium behind a movable bed with the patient. The attention of several hundred students was centered on this stocky "Bajuvarian" figure. With his head bent forward, rocking his heavy body in oversized shoes, he read the history himself. He interrupted the reading only for highlights of his own choosing. His interpretation of the patient's complaints was of great didactic value. He examined the patient meticulously or let the student take part, remembering Goethe's words, "The most difficult of all is that which you consider the easiest, namely to see with your own eyes what is in front of you." Dr. Müller explained that he diagnosed disease of the patient from symptoms, which are the expression of the deranged functions of organs. Thus he enabled the listener to build up a diagnosis by his own reasoning. He never attempted to force his students to a rigid scholastic interpretation by quoting questionable percentages of symptoms in similar cases. Through the simplicity of his diction and his sincerity, combined with an unusual knowledge of medical literature, Müller created in the lecture room an atmosphere which made him a great medical teacher.

The present-day methods of teaching in our medical schools are different. The chief of the department organizes the teaching schedule of his department. He himself selects only a few topics of his own preference. He delegates the main teaching to the younger instructors, who train the students systematically in small groups. There is no question but that a more detailed study is accomplished and greater factual knowledge acquired by the average student. Such a method is not unlike training a football team. The multiple variations of the "plays" and the details are called by the coaches (instructors) and then applied in the field. There is no question but that the medical student acquires with this type of training much factual knowledge, culminating in good marks in the final examination. One cannot be so sure whether a medical student of Dr. Müller's lectures would be able to produce as good marks in the state boards as a student trained by our medical schools. But the students of Dr. Osler and Dr. Müller, impressed by their masters' handling of the sick and by their constructive reasoning, did carry away example-setting experience which is just as important to the life of a physician as factual knowledge.

Friedrich von Müller visited the United States twice. He gave lectures and held clinics at different universities and towns. On his second visit he was invited to deliver the dedication address of the new University Hospital in Rochester, New York (1926). He admired Abraham Flexner and W. H. Welch in their task of reforming American medical schools. Müller himself was not a convinced supporter of a rigid full-time system. He thought that the professor should have the opportunity to see private patients in his office. In a letter to his friend, Graham Lusk, Müller quoted the New Testament: "Thou shall not muzzle the mouth of the ox that treadeth out the corn."

Dr. Müller always welcomed American co-workers at the Munich clinic and in its laboratories. American physicians who worked with him later became outstanding clinicians in their country.

Only a few may be named: George Fahr, L. Rowntree, Kenneth Robinson, George Draper, D. Van Slyke, W. McCann, Ralph Major, E. DuBois, D. Barr, and Cecil Watson.

Müller never imposed a theme for scientific research upon an investigator. He liked to quote K. Piloty, the famous head of the Royal Academy of Painting in Munich: "I can teach you how to paint, but the painting you must do yourself." Such an attitude made Friedrich von Müller the great personality his pupils will never forget.

BOOK REVIEWS

LOW-FAT COOKERY. *By Evelyn S. Stead and Gloria K. Warren. $3.95, pp. 184, cloth, McGraw-Hill Book Company, Inc., New York, 1956.*

The publication of *Low-Fat Cookery* comes at a time when interest in low-fat diets is at an all-time high. This book has been especially written for the housewife who is preparing meals for a family in which one or more members are on diets restricted in fat. The authors of the book are well qualified and aim to convince those who use the book that it is possible to prepare palatable food with little or no fat.

Ten chapters of the book are divided as most cook books are, under such subjects as appetizers and hors d'oeuvres, soup, meat, fish and poultry, cheese and cheese spreads, salads and salad dressings, sandwiches, sauces, vegetables, desserts and beverages. The eleventh chapter discusses breakfasts and the many ways of providing variety. Suggestions for the preparation of eggs, pancakes, hot breads and a variety of toasted breads are given.

The recipes are easy to follow and in every instance the yield is given. The total fat content of the recipe is given as well as the individual serving. Figures used in the calculations are from authoritative sources. One cannot help but find among the many recipes some new and original ideas, and there are approximately 150 of them. Among the helpful suggestions will be found a recipe for mayonnaise. Another for salad dressing and one for French dressing will add zest to the many salads suggested. The fat content of these dressings varies from 0.0 to 0.4 gm. per tablespoon.

Sample weekly menus averaging 25 gm. fat to 50 gm. per day are found in the Appendix. Analysis of the menus shows the amount of calories, protein, fat, carbohydrate, calcium, phosphorous, iron, vitamin A, thiamin, riboflavin, niacin and ascorbic acid. Comparison is made with the normal requirements as set up by the National Research Council in the Recommended Dietary Allowances.

Suggestions for the use of the book are given by two physicians. They say that "the present book is designed primarily for well people who wish to eat less fat, either to control weight or in the belief that they will then be less subject to vascular disease. Sick people should not use this book without the guidance of a physician." Weight loss is inevitable for those who use the recipes and the sample weekly menus which average between 1,600 and 1,800 calories.

Persons with diabetes are included among those who might use the book to advantage. A word of warning should be given to those whose diets are restricted in calories. Young persons and those who are engaged in very active occupations are permitted more calories in their diets and the carbohydrate allowance is usually more liberal. The weekly menus given in the Appendix average daily approximately 270 gm. carbohydrate. With proper instruction the diabetic person whose carbohydrate allowance is adequate could use the suggested menus and recipes. However, a table showing the carbohydrate content of the foods used in the recipes would enhance the value of the book to the diabetic.

Trade names of the products are used freely throughout the book. Some products are available only in certain areas of the country. Without the product named, the recipe requiring it would be useless. While addresses and instructions are given on how to obtain the product one feels that too much emphasis has been placed on the use of specific manufactured products.

The above criticism does not lessen the value of the book. Many persons may be able to obtain the products mentioned, and the wish of the authors "that cooking with less fat can be painless and even pleasant" may be fulfilled.

BIOCHEMISTRY. *By Abraham Cantarow, M.D., and Bernard Schepartz, Ph.D. $12.00, pp. 867, second edition, W. B. Saunders Company, Philadelphia, 1957.*

The second edition of Cantarow and Schepartz' *Biochemistry* is organized in much the same way as the earlier edition of this text. It is designed primarily for the first-year student of medicine and emphasizes the integration of biochemical processes "with the regulation and co-ordination of various aspects of metabolism." In most instances, the authors do not attempt to present the fundamental organic and physical chemistry which underlies the biochemical reactions which are discussed. From the point of view of most students of clinical medicine, this decision is probably a happy one and results in an unusually readable book. The tabular material, structural formulas, and reaction sequences are fairly extensive and clearly presented. Diagrams emphasizing the interrelationships and interconversions in the field of intermediary metabolism are helpful. This is an excellent book on basic biochemistry for the student of clinical medicine: It is not a book on the biochemical aberrations of specific disease states. While it is written primarily for the undergraduate student of medicine it offers the physician a reasonably up-to-date and thorough presentation of a rapidly expanding field.

ABSTRACTS

Annotation: DIABETES MELLITUS AND ANAESTHESIA. Lancet *1*:465, March 2, 1957.

Preferable preparation of the diabetic patient for general anesthesia is considered to be the intravenous administration of glucose and the administration of insulin in divided doses every four to six hours.

Annotations: DIABETICS AND DRIVING. Lancet *1*:310, Feb. 9, 1957.

This annotation presents a discussion of the legal aspect of auto accidents in which hypoglycemic attacks in diabetes are the cause of the mishap.

Annotations: GLUCOSE-TOLERANCE TESTS. Lancet *2*:33, July 6, 1957.

The measurement of glucose disposal by the increment index was found to be more valid than by the total index.

The effect of age on glucose-tolerance test values is discussed.

Annotations: TOLBUTAMIDE. Lancet *1*:259-60, Feb. 2, 1957.

Evidence at present suggests that tolbutamide is considerably less dangerous than carbutamide, but not completely innocuous since it may cause leukopenia. The mechanism of action of the sulfonylureas is discussed briefly and compared with that of insulin.

Annotations: TUBERCULOSIS AND DIABETES. Lancet *2*:131-32, July 20, 1957.

Pulmonary tuberculosis was found to be more common in diabetes than in the general population. The two disorders are considered to be antagonists.

Baird, C. W.; and Bornstein, J. (Dept. of Biochemistry, Univ. of Melbourne, Australia): PLASMA-INSULIN AND INSULIN RESISTANCE. Lancet *1*:1111-13, June 1, 1957.

A description of a method for extracting insulin from plasma. Cases of diabetic coma are presented in which insulin was present in the plasma, but its activity was masked by antagonists. In this method of extraction the antagonists were fractionated from the insulin. Diabetics are classified into two groups: those with available plasma-insulin and those without available plasma-insulin.

Baron, D. N.; and Oakley, Celia M. (Royal Free Hosp., London, England): A RAPID BLOOD-SUGAR SCREENING METHOD. Lancet *1*:326, Feb. 8, 1957.

Good results are reported on estimating blood sugar levels in serum or plasma after separation of fingerstick blood. Tes-Tape is used as the indicator.

Barta, L. (First Dept. of Pediatrics, Budapest Univ. Med. Sch., Hungary): CARBUTAMIDE IN JUVENILE DIABETES. Lancet *1*: 1090, May 25, 1957.

A study of sixteen diabetics between ages of six and thirteen years who were tried on carbutamide. Results showed blood sugar levels were not lowered and that combining the drug with insulin proved unsatisfactory.

Barta, L.; and Simon, G. (First Dept. of Pediatrics, Budapest Univ. Med. Sch., Hungary): EFFECTS OF CARBUTAMIDE ON CARBOHYDRATE METABOLISM IN DIABETES. Lancet *1*:1249-50, June 15, 1957.

A report on experiments on rabbits in which the effect of carbutamide on the rise of blood sugar levels following a fall induced by insulin was studied. Results showed that there was a significant outflow of glucose from the liver in spite of carbutamide treatment.

Bastenie, P. A.; Franckson, J. R. M.; De Meutter, R.; Demanet, T. C.; and Conrad, V. (Univ. Dept. of Medicine, St. Pierre Hosp., Brussels, Belgium): METABOLIC EFFECT OF CARBUTAMIDE IN SELECTED DIABETICS. Lancet *1*:504-07, March 9, 1957.

A report on eighteen diabetics (sixteen with onset of disease in middle age) controlled on BZ-55. Intravenous glucose-tolerance tests, intravenous insulin tests and combined intravenous glucose and insulin tests were given before treatment, after initial treatment and after protracted treatment.

Results showed BZ-55 had no insulin-sparing action and did not increase activity or secretion of insulin; but it did decrease the usual large output of glucose from the liver during insulin-induced hypoglycemia.

Thus the action of BZ-55 is probably best explained by the inhibition of hepatic glucose-6-phosphatase.

Bauer, Heinz; Flanagan, John F.; and Sheldon, Walter H. (Emory Univ. Sch. of Med., Dept. of Path., Emory Univ., Georgia): THE EFFECTS OF METABOLIC ALTERATIONS ON EXPERIMENTAL RHIZOPUS ORYZAE (MUCORMYCOSIS) INFECTIONS. Yale J. Biol. & Med. *29*:23-32, September 1956.

To test if experimentally induced metabolic alterations in a previously normal animal can change the fungus from a saprophyte to a pathogen, normal rabbits were inoculated with fungus at various times preceding the production of diabetes by alloxan administration.

Normal rabbits inoculated with fungus show only rare, minute lesions at the site of inoculation, but fungus cultures from the tissues at autopsy are positive for as long as thirteen days after inoculation. It appears, therefore, that the fungus persists in the tissue of the metabolically normal animal, and that the host cannot readily destroy the agent. An aggressive fungus infection is, however, precipitated when acute alloxan diabetes is induced in rabbits that have been inoculated with fungus either one or three days before alloxan injection. In this experiment, all but one rabbit developed extensive lesions in one or several sites.

Bell, E. T. (Dept. of Path., Univ. of Minn. Med. Sch., Minneapolis, Minn.): ATHEROSCLEROTIC GANGRENE OF THE LOWER EXTREMITIES IN DIABETIC AND NONDIABETIC PERSONS. Am. J. Clin. Path. *28*:27-36, July 1957.

Among 50,184 nondiabetic and 1,878 diabetic persons, this autopsy study summarizes important aspects of atherosclerotic gangrene occurring after the age of forty years. The study covers a forty-five-year period, from 1911 through 1955. There were 186 instances of atherosclerotic gangrene of one or both lower extremities in the nondiabetic and 393 in the diabetic patients. The study demonstrates that atherosclerotic gangrene is fifty-three times more frequent in diabetic men over sixty and seventy-one times more frequent in diabetic women. Two-thirds of the cases of gangrene in men less than eighty years of age

and one-fourth of those in men more than eighty years of age were the result of diabetes, and 80 per cent of the gangrene in women resulted from diabetes. There was no correlation between the development of the gangrene and the need for insulin for the known duration of the diabetes.

Gangrene was unilateral in 74 per cent of the diabetic and 79 per cent of the nondiabetic patients. Infection was an important factor in diabetic gangrene, but unimportant in the nondiabetic patients. The period of survival after amputation was longer among the diabetic patients.

Hyalinization of the juxtaglomerular segment of the afferent-glomerular arteriole was found in 87.3 per cent of the diabetic and in 10 per cent of the nondiabetic persons. Advanced hyalinization of this afferent arteriole indicates either frank diabetes or a prediabetic state. Furthermore the presence of intercapillary glomerulosclerosis was recognizable among 48 per cent of the diabetic patients on microscopic examination. There was no significant difference in the frequency of hypertension in the two groups, both showing an incidence of blood pressure below 150 systolic and 90 diastolic in approximately 40 per cent with gangrene. Blood pressure of 180 systolic and 90 diastolic was present in 29 per cent of those with diabetes and 25 per cent of those without diabetes.

Boon, Wong Hock. (Pediatric Unit, Genl. Hosp., Singapore): DIABETES MELLITUS IN A TWO-MONTH-OLD CHINESE BABY. Arch. Dis. Childhood 32:197-98, June 1957.

A case of diabetes, diagnosed by attraction of ants to wet diapers as well as by glycosuria, weight loss, and elevated blood sugar, is reported in a two-month-old Chinese infant. The treatment regimen consisted of 2 units of soluble insulin before each feeding and later a single daily dose of 7 units of insulin-zinc suspension. Follow-up glucose tolerance curves were diabetic in nature.

Butterfield, W. J. H.; Camp, J. L.; Hardwick, C.; and Holling, H. E. (Med. Res. Council; Univ. Virginia Med. Sch.; Guy's Hosp., London; Med. Res. Council): CLINICAL STUDIES OF THE HYPOGLYCAEMIC ACTION OF THE SULFONYLUREAS. Lancet 1:753, 56, April 13, 1957.

A series of twenty-nine diabetics were treated with sulfonylureas for seven days. The effects on various carbohydrate-tolerance tests, peripheral blood flow, and hypoglycemic response to glucagon administration were studied. In twenty-two of the twenty-nine the fasting blood sugar fell more than 15 per cent. The only significant finding after treatment was that the blood sugar level fell below the fasting level at sixty and ninety minutes.

Constam, George R. (Zurich, Switzerland): INSULIN-ZINC SUSPENSIONS (LENTE INSULINS) IN THE TREATMENT OF DIABETES MELLITUS. Northwest Med. 56:1023-30, September 1957.

The author reviews the development and chemistry of Lente insulins. He feels that the action of insulin-zinc suspensions does not differ from other insulins, but that because of simple composition and reliable action they should be used for diabetics who start treatment with long or intermediate-acting insulins. Mixing of Lente insulins is acceptable, but the author feels that combination with regular insulin should be avoided. He notes that allergic skin reactions occur even with insulin-zinc suspensions.

Constam, George R. (Zurich, Switzerland): SULFONYLUREA COMPOUNDS IN THE TREATMENT OF DIABETICS. Northwest Med. 56:1155-66, October 1957.

The author reviews the development of the oral sulfonylureas in treatment of diabetes and current theories of their activity. He concludes that the compounds are useful but still unproved. The review has an extensive bibliography.

Daly, J. J. (Royal Hosp., Sheffield, England): ADRENAL ADENOMATA IN ELDERLY DIABETICS. Lancet 2:710-11, Oct. 6, 1956.

The incidence of adrenal adenomata is reported from a series of 789 necropsies. Thirty-one adrenal tumors were found and six of these had a history of diabetes of short duration. The mechanism of the diabetes is discussed.

Davies, J., and Lacy, Paul E. (Depts. of Anat. and Path., Washington Univ., St. Louis, Mo.): OBSERVATIONS ON THE FAILURE OF INSULIN TO PASS FROM THE FETUS TO THE MOTHER IN THE RABBIT. Am. J. Obst. & Gynec. 74:514-17, September 1957.

A study of the effects on the maternal blood sugar level of large doses (240 units) of insulin injected into the blood stream of fetal rabbits close to term. Of five animals successfully studied, no alteration took place in the maternal blood sugar level over a two-hour period in three animals and a slight fall occurred in two animals. The lowest recorded blood sugar levels in the latter two animals were 66 mg. per cent and 46 mg. per cent, respectively. They were higher than that produced in a control animal following the intravenous injection of 2 units of insulin (27 mg. per cent within one hour). In eight other animals a definite hyperglycemia was shown to follow the intrafetal injection of insulin, a phenomenon that was observed to be associated with damage to the placenta. It was concluded that no evidence was obtained to support the view that insulin can pass across the placenta from the fetus to the mother in the rabbit.

DeBonis, C. S. (Dept. of Int. Med., Geisinger Mem. Hosp. and Foss Clin., Danville, Pa.): INSULIN RESISTANCE AND SENSITIVITY. Bull. Geisinger Mem. Hosp. & Foss Clinic 9:76-80, May 1957.

Insulin resistance may occur at any age, and may last a few days, months, or years. Both sexes are equally vulnerable. In addition, there does not seem to be any significant incidence of any associated disease or pathologic process occurring in these people. Resistance can occur in nondiabetics. This has been observed repeatedly in psychiatric centers using insulin shock therapy.

A favorable termination of resistance will occur in 20 to 40 per cent of patients. This result is attributed to no therapeutic procedure other than good diabetic management and time. Insulin resistance will generally wear itself out in about thirty-six months.

Disregarding management of the concomitant disease, therapy per se resolves itself into the prevention of ketone acidosis. Since the dominant action of insulin is to facilitate the entry of glucose into the cycle of carbohydrate metabolism, if the metabolic pathway is open it should be possible to give sufficient insulin to produce this effect. Adequate insulin should overcome the immunologic inactivation, the excessive rapid destruction, the hormonal antagonism, or the unknown factor that renders its action ineffective. Too much insulin might be required, posing the danger of severe hypoglycemic reactions should the patient's status suddenly change. Nevertheless, the

patient can usually be kept between coma and insulin reactions by meticulous observation until the temporary state of insulin resistance disappears.

Dickey, Robert F. (Danville, Pa.): DERMAL MANIFESTATIONS ASSOCIATED WITH DIABETES MELLITUS. Pennsylvania M. J. 60:857-63, July 1957.

Although a few skin lesions such as xanthoma diabeticorum and necrobiosis lipoidica diabeticorum are practically pathognomonic of diabetes mellitus, most of the dermal lesions so frequently associated with diabetes mellitus are not specific but rather are strongly suggestive of diabetes. Such nonspecific dermal manifestations of diabetes mellitus include vulvitis, vaginitis, balanitis, intertriginous dermatitis, moniliasis, ecthyma, carbuncles, furunculosis, and peripheral vascular occlusive syndromes. Such skin diseases frequently are associated with debilitating systemic illnesses, and, as such, each of these dermatoses requires that the physician carry out diligent etiologic analyses in order properly to diagnose and treat the underlying internal disease. These various dermatoses should serve as "red flag" diagnostic warnings of possible associated diabetes mellitus.

Editorial: MANAGEMENT OF THE DIABETIC PATIENT—A NEW LOOK. New England J. Med. 256:619, March 28, 1957.

Comments on the opening of the Hospital Teaching Clinic associated with the New England Deaconess Hospital by the Diabetes Foundation, Inc. Minimal care for ambulatory diabetics permits instruction and regulation at reduced cost.

Editorial: ORAL TREATMENT OF DIABETES. New England J. Med. 256:89, Jan. 10, 1957.

Reviews the experience with carbutamide and tolbutamide, stressing the need for caution in view of some of the toxic effects now reported.

Engleson, Gunnar; and Lehmann, Orla (Pediatric Dept., Univ. Hosp., Lund, Sweden): B.Z.-55 IN JUVENILE DIABETES. Lancet 2:786, Oct. 13, 1956.

BZ-55 was tried in juvenile diabetics and not-too-encouraging results are reported.

Engleson, Gunnar; and Zetterqvist, Per (Lund, Sweden): CONGENITAL DIABETES MELLITUS AND NEONATAL PSEUDODIABETES MELLITUS. Arch. Dis. Childhood 32:193-96, June 1957.

Two infants, ages five days and eleven days respectively when glycosuria and elevated blood sugars were discovered, are discussed. Insulin was given in both cases and discontinued after three months in one and seven months in the other.

One child has maintained normal blood sugars without insulin for more than three years. Follow-up glucose tolerance curves or blood sugar levels have been normal in both children. The author favors the theory that "neonatal pseudodiabetes" may have resulted from carbohydrate disturbance of a steroid diabetic character as a result of placental dysfunction.

Fagerberg, Sven-Erik (Sahlgren's Hosp., Univ. of Gothenburg, Sweden): STUDIES ON THE PATHOGENESIS OF DIABETIC NEUROPATHY: III. DIABETIC NEUROPATHY IN RELATION TO DIABETIC VESSEL COMPLICATIONS. Acta med. scandinav. 157:401-06, June 5, 1957.

On the assumption that diabetic neuropathy, similar to retinopathy and nephropathy, is elicited from specific angiopathy, cases of diabetic neuropathy in different age-groups have been examined with respect to the occurrence of specific vessel changes and so-called arteriosclerotic vessel manifestations. The investigations suggest that neuropathic cases do not form a special group, but rather have the same pattern as diabetic nephropathy and retinopathy.

Fahlgren, H.; Andersson, B.; and Lundmark, C. (Med. Dept. and Dept. Path., Södersjukhuset, Stockholm, Sweden): PROLONGED UNCONSCIOUSNESS WITH AN ATTACK OF HYPOGLYCEMIA: CASE WITH FATAL OUTCOME AND ADVANCED CEREBRAL CHANGES. Acta med. scandinav. 158:97-101, Aug. 13, 1957.

A thirty-seven-year-old man was admitted to the hospital, unconscious and with hypoglycemia of unknown duration. Though blood sugar returned to normal within a few hours, unconsciousness persisted for six weeks with a fatal outcome. Autopsy revealed severe cerebral damage, with numerous small areas of necrosis and hemorrhagic foci up to the size of a hen's egg. Origin of these cerebral lesions is discussed, and they are interpreted as manifestations of a posthypoglycemic encephalopathy.

Gerritzen, F.; Noach, E. L.; van Wijhe, M.; and Valk, L. E. M. (Univ. of Leyden, Leyden, Holland): INSULIN DEFICIENCY AND DIABETES. Acta endocrinol. 25:91-100, May 1957.

There is no experimental or clinical evidence to support the commonly accepted belief that insulin deficiency is the cause of late diabetic complications. Until this has been proved, it is more correct to speak of experimental insulin deficiency and not to use the expression "experimental diabetes" since it is possible that insulin deficiency is only a part of the clinical syndrome: diabetes.

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de Graeff, J.; and Lips, J. B. (Dept. Endocrine Dis. and Metab., and Dept. Med., Univ. Hosp., Leyden, Holland): HYPERNATRAEMIA IN DIABETES MELLITUS. Acta med. scandinav. 157:71-75, March 10, 1957.

In two patients with unregulated diabetes mellitus, without severe acidosis, hypernatremia and hyperchloremia as manifestations of extracellular hypertonicity were found. In both patients the clinical picture resembled diabetic precoma in some aspects. Treatment with large quantities of water resulted in complete clinical recovery. The pathogenesis is considered to be the following: (1) osmotic diuresis with the resultant loss of water, resulting in extracellular hypertonicity; (2) lack of thirst preventing the compensation of the water loss; (3) retention of sodium chloride by the kidneys due to relative peripheral circulatory failure of the heart, caused by a reduction of the circulating blood volume.

Günther, Otfried (The Home for Diabetics and the Inst. for Res. and Therapy of Diabetes Mellitus, Garz and Karlsberg, Germany): HEPATITIS AND DIABETES. Ztschr. ges. inn. Med. 11:656-60, July 15, 1956.

The incidence of hepatitis in diabetes was studied via the case histories of 10,000 diabetic inpatients during the twenty-five-year-period 1930-1955. While in 401 instances hepatitis had preceded the diabetes by various time intervals, hepatitis had developed after the onset of the first manifestations of diabetes in 755 instances, a significantly higher number. Of these patients, 693 (91.7 per cent) were on insulin treatment while the proportion of insulin-treated patients in the whole group was somewhat lower (78.3 per cent). Moreover, the incidence of hepatitis was highest during the first year of known diabetes. The author concludes from these data that the surprisingly great incidence of hepatitis was not so much due

to a doubtful greater susceptibility of the diabetic to infections in general or his predisposition to liver diseases, but to the risk the insulin-treated patient runs of catching the virus of homologous serum hepatitis. The high incidence shortly after the onset of diabetic manifestations is related to the fact that most patients are hospitalized during that early phase of the disease and that the possibility for transmission of the hepatitis-virus is greatest in the hospital or physician's office. At home, insulin syringes and needles are used solely by the patient himself. To test his conclusion, the author changed in 1952 the sterilization technics in his institution from boiling for twenty minutes to hot air sterilization at 160° for sixty minutes. The incidence of hepatitis decreased significantly after 1952. Thus, only twenty-one cases of hepatitis were observed during the two-year-period 1952-53, compared with seventy-three cases during the years 1949-50 with an about equal number of patients newly placed on insulin during these two periods.

The unusually high incidence of hepatitis in the case material of this German study must be viewed against the background of the poor hygienic and nutritional conditions in Germany during a great part of the observation period, which includes the war and postwar years. It therefore may not be comparable with conditions in the United States. Nevertheless, the study may offer an explanation for the great discrepancy in various reports about the relationship between diabetes and liver disease.

Hackedorn, Howard M. (Seattle, Wash.): TREATMENT OF DIABETES WITH SULFONYLUREAS. Clin. Med. *4*:599-601, May 1957.

A new approach to the study of carbohydrate metabolism and the treatment of diabetes, with instances of its success and failure analyzed.

Hallay, Leo I. (Fort Blackmore, Va.): EXPERIENCES WITH NICOTINIC ACID IN DIABETES MELLITUS. Virginia M. Month. *84*:403-05, August 1957.

Symptomatic relief of diabetes in the cases reported in this paper suggests, according to the author, the following: (1) Nicotinic acid, as others have reported, exerts a mild insulin-sparing action; (2) the amount of insulin given to patients with diabetes can be substantially reduced by simultaneous administration of nicotinic acid; (3) mild cases of diabetes may obtain symptomatic relief when treated with nicotinic acid only; (4) while insulin treatment is essential in severe cases of diabetes mellitus, the development of arteriosclerosis, which is seen to take place rather early even in patients treated with insulin, can be slowed down by simultaneous administration of nicotinic acid.

Haunz, Edgar A. (Grand Forks Clin., Grand Forks, N. D.): CLINICAL EVALUATION OF LENTE INSULIN IN ONE HUNDRED NINE DIABETIC PATIENTS. J.A.M.A. *159*:1611-18, Dec. 24, 1955.

Insulin-zinc suspensions exist in two basic physical forms—amorphous and crystalline. The amorphous precipitate forms when the *p*H is adjusted rapidly upward during preparation. The resultant suspension exists in a form more rapidly absorbed, having a time-action potential of about twelve to eighteen hours. This preparation is called Semilente insulin. The crystalline form, on the other hand, results when insulin crystals are suspended in a solution of a zinc salt and remain undissolved in the *p*H range of 5-8, provided substances that interact with the zinc are not present. The crystalline form is termed Ultralente insulin, a single injection of which may be capable of acting as long as ninety-six hours.

It is readily apparent that the time action of neither Semilente nor Ultralente insulin is completely satisfactory. However, the time-action potential can be varied at will simply by admixture of different quantities of these two basic insulin-zinc suspensions.

Six of the total of 109 patients, having no allergy to previously used insulins, exhibited definite localized allergy, such as redness, swelling, and itching, at sites of Lente insulin injections. In one instance this phenomenon disappeared in the second week with Lente insulin therapy. In three instances the allergy disappeared when special beef Lente insulin was substituted, but in the remaining two cases allergy persisted even after substitution of special beef Lente insulin; however, the allergy disappeared upon resuming use of the previous (Isophane) insulin. In all groups, a total of twenty-four patients (22 per cent) exhibited mild to severe allergic reactions during therapy with previously used insulins, while only fifteen patients (14 per cent) exhibited similar reactions to Lente insulin.

Lente insulin appears to be somewhat more efficient than Isophane insulin on a unit for unit basis in the majority of cases. In converting a patient from Isophane to Lente insulin, these results suggest that one should begin the latter at slightly lower dosage than the current dosage of Isophane.

Admixture of Lente and unmodified insulin (zinc-insulin crystals) appears to be efficient and practicable, at least in the more stable cases of diabetes mellitus, but further studies are necessary to clarify its limitations. Until further investigation on mixtures is forthcoming, it is suggested that no mixture should contain more unmodified insulin than Lente insulin, to avoid disrupting the buffer mechanism that preserves the independent time-action potential of Lente insulin. Insulin made from zinc-insulin crystals is the unmodified insulin preferred for admixture at the present time.

Heijkenskjöld, F.; and Sandberg, L. (Dept. Clin. Chem. and Med. Dept., Karolinska Sjukhuset, Stockholm, Sweden): CARBOHYDRATE EXCRETION IN RENAL GLYCOSURIA; INVESTIGATION OF TWO CASES. Acta med. scandinav. *157*:141-47, March 25, 1957.

Investigation was made of reducing substances in the urine of two patients with renal glycosuria. It showed the presence of glucose, galactose, amino sugars and lactose. Increased amounts of fluorescing substances and amino acids in the urine suggest the possibility that the enzymatic deficiency of the renal tubules is not specific for carbohydrates.

Helps, E. P. W. (Dept. of Med., Johns Hopkins Hosp., Baltimore, Md.): SENILE CONFUSION AND THE BLOOD-SUGAR LEVEL. Lancet *1*:138-39, Jan. 19, 1957.

A report of five cases of elderly diabetics with cerebral arteriosclerosis in which symptoms of hypoglycemia occurred in the presence of elevated blood sugar levels. In each instance symptoms improved as the blood sugar level was allowed to rise.

Hernberg, Carl A. (Med. Dept., Maria Hosp., Helsingfors, Finland): ORAL TREATMENT OF DIABETES MELLITUS WITH THE SULFONAMIDE BZ-55. Acta endocrinol. *23*:281-88, November 1956.

Sixty-two patients with diabetes mellitus not exceeding five years' duration were treated with the sulfonamide BZ-55. In

fifty-two out of sixty-two cases the effect was good insofar as the reduction or disappearance of glucosuria, or need for insulin were concerned. In six cases in which the drug had no effect, patients were younger than forty-five years. Twenty-seven out of forty-one patients treated with insulin were able to do without it, following courses of BZ-55. The favorable effect on the diabetes after cessation of treatment with BZ-55 lasted for different periods varying from four to eighty-four days, irrespective of the age of the patients or duration of the diabetes. Acute infections as well as cortisone in some cases caused a temporary rise in the sugar values. The BZ-55 mechanism is discussed.

Heskel, Milton M. (Dept. of Endocrin., A. Einstein Med. Ctr., Philadelphia, Pa.): HISTOPATHOLOGIC STUDIES OF THE PITUITARY GLAND IN DIABETES MELLITUS. J. Albert Einstein M. Center 5:189-94, June 1957.

There appeared to be a characteristic shift toward maximal granulation in the mucoid cells of the anterior pituitary gland in all diabetic patients in this study who had intercapillary glomerulosclerosis and in young diabetics without this complication. Since Pearse had reported similar results in patients with adrenal cortical hyperfunction, maximal-intermediate counts were performed on three cases of Cushing's disease due to adrenal cortical hyperplasia. These demonstrated maximal cell predominance in addition to hyaline basophils.

The similarity between the maximal-intermediate ratios in certain patients with diabetes mellitus and patients with adrenal cortical hyperfunction is most intriguing since there has been experimental and clinical evidence suggesting a relationship between adrenal cortical hyperfunction and the production of intercapillary glomerulosclerosis and diabetic retinopathy. Accordingly, sections of adrenal glands from nine patients with diabetes mellitus complicated by intercapillary glomerulosclerosis were studied. Six of these nine patients had adrenal glands exhibiting nodular cortical hyperplasia. In contrast, only one of seven diabetics of various ages without intercapillary glomerulosclerosis had adrenal cortical nodular hyperplasia.

Hill, M. M. (Barrister-at-Law): UNDER THE INFLUENCE OF INSULIN. Lancet 1:319-20, Feb. 9, 1957.

Presentation of a court case of a diabetic involved in an auto accident because of hypoglycemic reaction.

Hoet, Joseph P. (Clinique Medicale, Hôpital St. Pierre, Louvain, Belgium): ADRENOCORTICAL FUNCTION IN INFANTS OF DIABETIC MOTHERS. Cold Springs Harbor Symposia 19:182-85, 1954.

Normal amniotic fluid is free of corticoids. Amniotic fluid of a diabetic woman treated with insulin from the fourth month of her pregnancy contained appreciable quantities of corticoids. The possible role of corticoids in a pathogenic syndrome resembling the Cushing syndrome in infants born to diabetic women is studied here. The presence of edema of the fetal placenta (even when there is Rh incompatibility) is associated with transitory diabetes during pregnancy.

The use of cortisone during pregnancy is able to provoke an imbalance in carbohydrate metabolism which alone may lead to fetal death.

Holten, C.; Lundbaek, K.; and Staffeldt, Ingrid (University Clin. of Internal Med., Kommunehospitalet, Aarhus, Denmark): AN INVESTIGATION OF THE ACTION OF CORTISONE AND PREDNISONE ON INTRAVENOUS GLUCOSE TOLERANCE. Acta. med. scandinav. 157:257-62, May 4, 1957.

Intravenous glucose tolerance tests were made in ambulatory patients suffering from diseases not known to influence carbohydrate metabolism. No amelioration of the glucose tolerance test was observed when two consecutive intravenous tests were performed. Two hours after the oral administration of cortisone or prednisone a decrease of the slope of the tolerance curve occurred. The decrease in the glucose tolerance is of the same order of magnitude after 20 mg. of prednisone as after 100 mg. of cortisone.

Irvine, R. E.; and Rowell, N. R. (Royal Victoria Infirmary, Newcastle Upon Tyne, England): COMA IN DIABETES WITH RENAL FAILURE. Lancet 2:1025-26, Nov. 17, 1956.

A case presentation of recurrent coma in a patient with diabetic nephropathy and renal failure; severe acidosis and hyperglycemia occurred without ketonemia and ketonuria.

Jaffe, Louis; Leach, Robert B.; and Salem, Edward S. (Wayne State Univ. Coll. of Med., Detroit, Mich.): LENTE INSULIN: A CLINICAL EVALUATION. J. Michigan M. Soc. 56:882-83, 918, July 1957.

Eighteen patients considered well controlled with various other insulins, were given a similar dose of Lente before breakfast. As expected, most of the patients (eleven) had a 4 p.m. low point. However, an important percentage (33 per cent) exhibited a 12 o'clock (before lunch) low point, indicating greater sensitivity to the Semilente component of the mixture. In general, these patients had somewhat higher fasting levels the next morning, indicating that in these circumstances a higher percentage of Ultralente in the insulin mixture administered would have been desirable. Five "brittle" diabetic patients, considered uncontrolled by all previous methods, were given equivalent Lente doses and followed for forty-eight hours. Two remained grossly uncontrolled and three exhibited distinct improvement. However, the inherent variability of control in these cases made the duration of study too short to lead to significant conclusions.

Jersild, M. (Hvidøre Hosp., Copenhagen, Denmark): INSULIN ZINC SUSPENSION—FOUR YEARS' EXPERIENCE. Lancet 2: 1009-13, Nov. 17, 1956.

The experience with 1,000 patients over a four-year period is presented. Changes in insulin dosage, type of mixtures of Lente, Semilente, Ultralente and crystalline insulins and the various effects are discussed. The data suggest that it is more difficult to obtain control with any of these insulins on diets with high caloric intake. The incidence of local reactions to insulin was found to be 0.8 per cent.

Jiménez Díaz, C.; Oya, J. C. de; and Rodríguez Miñón, J. L. (Dept. of Physiol., University of Madrid, Madrid, Spain): MECHANISM OF THE PROTECTIVE ACTION OF ADRENALIN ON ALLOXAN DIABETES. Bull. Inst. M. Res. 9:41-47, January-March 1956.

The injection of alloxan after clipping the renal pedicles in the dog gives rise to a remarkable increase in blood sugar and lactic acid levels. The increase is similar to that induced by injecting adrenalin. Constriction would, therefore, induce the release of adrenalin by the adrenal glands. The injection of sodium lactate in doses of 2 gm. per kg. of body weight results in hyperlactacidemia and prevents alloxan diabetes in the same way as renal clipping. The mechanism of protection would, therefore, take place in the following order: clipping, adrenalin release, hyperlactacidemia. The increase in lactic acid

would, in the last analysis, be responsible for counteracting the diabetes-inducing action of alloxan by means of a mechanism as yet unknown.

Knick, Bernhard; and Emrich, Karl (Dept. of Medicine, University of Mainz, Germany): REVERSIBILITY OF ABNORMAL LIVER FUNCTION TESTS IN CHRONIC LIVER DISEASES (CHRONIC HEPATITIS, CIRRHOSIS) BY SULFONYLUREA MEDICATION (D860). Klin. Wchnschr. *35*:812-14, Aug. 15, 1957.

The authors attempted to evaluate the effect of the hypoglycemic sulfonylureas on the liver function in patients with chronic hepatitis and with cirrhosis of the liver. This was done because, on the one hand, the hypoglycemic sulfonylureas (carbutamide as well as tolbutamide) have been found to increase the liver glycogen level and because, on the other, an augmentation of the liver glycogen content is thought to have protective or therapeutic effect in chronic liver disease. The lack of any evidence of liver damage due to the sulfonylureas seemed to justify this trial. Six patients each with chronic hepatitis and with cirrhosis were treated with 3 gm. tolbutamide daily for periods from nineteen to thirty-four days. The clinical diagnoses were confirmed by laparoscopy. No data, however, are given about the clinical condition of the various patients prior to and after the treatment. Instead, results of a series of liver function tests done prior to and after the treatment period are reported. Appreciable amelioration occurred in ten of the twelve cases in regard to total serum bilirubin, Takata reaction, thymol turbidity and bromsulfalein retention. The total serum protein was within the normal range in all cases at the onset and did not show significant changes. The electrophoretically determined protein fractions showed in most cases a small increase in the albumin and a small decrease in the λ globulin fraction, but neither had been markedly abnormal prior to the sulfonylurea treatment. The α_1, α_2 and β globulin showed small fluctuations in either direction. The authors caution rightly against therapeutic enthusiasm about this medication until more extended studies have become available.

Kohn, J. (Queen Mary's Hosp., Roehampton, London, England): A RAPID BLOOD-SUGAR SCREENING METHOD. Lancet *1*:213, Jan. 26, 1957.

A preliminary report on the use of glucose enzyme strips as a rapid test in estimating blood sugar levels. Good correlation between blood sugar level and intensity of color on the strip is reported.

Kohn, J. (Queen Mary's Hosp., Roehampton, London, England): A RAPID METHOD OF ESTIMATING BLOOD-GLUCOSE RANGES. Lancet *2*:119-21, July 20, 1957.

A rapid method of estimating blood-glucose ranges by the use of enzyme test strips—Clinistix—is discussed. Only fresh finger-stick blood is used and the test is completed in less than one minute. The results indicate only a range and not absolute values. The quantitative estimations appear more accurate on blood than on urine.

Lawrence, R. D. (British Diabetic Association, London, England): PRESCRIPTIONS FOR DIABETICS. Lancet *1*:268, Feb. 2, 1957.

Insulin can now be prescribed for three months or more in England.

Lewis, J. G.; and Stephens, P. J. (Diabetic Dept., The Royal Free Hosp., London, England): A RAPID METHOD OF ESTIMATING BLOOD-GLUCOSE RANGES. Lancet 2:240-41, Aug. 3, 1957.

By use of enzyme test strips and finger blood a bedside determination is reported. The results give a range of blood sugar rather than an absolute value.

McKendry, J. B. R.; Kuwayti, Kamal; Sagle, Lloyd Alexander (Ottawa Civic Hosp., Ottawa, Ontario, Canada): EXPERIENCE WITH TOLBUTAMIDE (ORINASE) IN THE MANAGEMENT OF 100 CASES OF DIABETES. Canad. M. A. J. *77*:429-38, Sept. 1, 1957.

In a series of 100 diabetics selected at random from hospital and clinic practice, 60 per cent responded favorably to tolbutamide and 40 per cent did not. One elderly male succumbed to hypoglycemia. (This case was previously reported in Canad. M. A. J. 572-73, April 1, 1957.) The authors' experience parallels that of other reports.

Martin, T. Sterling (El Paso, Texas): THE DUALITY OF DIABETES MELLITUS AND ITS RELATIONSHIP TO THE USEFULNESS OF THE NEW ORAL "ANTIDIABETIC AGENTS." Southwestern Med. *38*:423-25, July 1957.

The author describes and compares two types of diabetes, i.e., primary insulin insufficiency and lipoplethoric diabetes. Following this description, he discusses action of Orinase and its use in both types. He concludes that Orinase is effective almost exclusively in the overweight diabetic over forty whose diabetes is of few years' duration, uncomplicated, and who is being carried on small to moderate doses of insulin, and leaves this profound thought with the reader: Whether to use it in an individual case in this group, particularly when further weight loss alone would suffice, will require good judgment by the physician.

Munro, I. B.; and Murray, D. (Dept. of Metabolic Diseases, Victoria Infirmary, Glasgow, Scotland): EFFECT OF CARBUTAMIDE ON SERUM-CHOLESTEROL LEVEL IN DIABETES MELLITUS. Lancet *2*:1083-84, Nov. 24, 1956.

The effect of carbutamide on serum-cholesterol levels in the blood was observed in six patients. A drop during the first few days of therapy was observed with a tendency to regain the previous level even if therapy is continued.

Oppé, T. E.; Hsia, David Yi-Yung; and Gellis, Sydney S. (Univ. of Bristol, England; Northwestern Univ. Med. School, Chicago, Ill.; Boston Univ. School of Med., Boston, Mass.): PREGNANCY IN THE DIABETIC MOTHER WITH NEPHRITIS. Lancet *1*:353-54, Feb. 16, 1957.

This is the report of a study of forty-one pregnancies in diabetic mothers with nephritis. The results confirm the high fetal mortality where maternal diabetes is complicated by nephritis. The results show greater likelihood of early intrauterine death and a tendency toward hyaline membrane formation following delivery.

Page, Otto C.; Hare, Robert L.; Stephens, John W.; and Holcomb, Blair (University of Oregon Med. School, Portland, Ore.): TOXICITY OF CARBUTAMIDE: REPORT OF A FATAL CASE OF BONE-MARROW DEPRESSION AND ANURIA. New England J. Med. *256*:74-76, Jan. 10, 1957.

The authors cite the incidence of side reactions in a group of forty-eight patients treated with carbutamide. The full case history is given for one patient who expired as a result of bone-marrow depression, anuria due to renal tubular degeneration and pseudomonas aeruginosa septicemia.

Ralli, Elaine P. (Coll. of Med., New York University-Bellevue Med. Center and Third Med. Div. of Bellevue Hosp., New York, N. Y.): THE MANAGEMENT OF ENDOCRINE CRISES.

M. Clin. North America 717-33, May 1957.

The author includes in this article a section on the management of the patient in diabetic ketosis, discussing the diagnosis and treatment and the chemical changes that occur in this crisis. She reiterates that only crystalline or quick-acting insulin should be used in patients in diabetic acidosis, and usually intravenous fluid therapy should be instituted simultaneously in the form of 5 per cent glucose in normal saline initially. She emphasizes the giving of potassium and warns against too rapid administration of potassium intravenously. There is also a brief discussion on hypoglycemia, its causes and treatment.

Report. INSULIN COMA IN A DRIVER. Lancet *1*:1345, June 29, 1957.

Report of a case of a diabetic who had an insulin coma while driving and was found guilty of driving under the influence of a drug by the court.

Robillard, Rosario; and Saucier, Robert (Saint Luke's Hosp., Montreal, Quebec, Canada): STUDY OF BLOOD CHOLESTEROL LEVELS IN OBESE DIABETICS USING PHENMETRAZINE (PRELUDIN). Canad. M. A. J. 76:498-99, Sept. 1, 1957.

Using Phenmetrazine as an anorexient in obese diabetics, the authors noted no consistent relationship between weight loss and blood cholesterol levels.

Rosenthal, F. D. (Royal Infirmary, Sheffield, England): ADRENOCORTICAL HYPERACTIVITY IN A PATIENT WITH BRONCHIAL CARCINOMA AND DIABETES MELLITUS. Brit. M. J. *2*:139-41, July 20, 1957.

The author reports a diabetic patient with bronchial carcinoma in which marked hyperexcretion of adrenocortical hormones was noted. In spite of severe steroid diabetes only a few of the features of Cushing's syndrome were observed. The possibility that marked adrenocortical activity was due to stimulation of the pituitary in response to the presence of the bronchial carcinoma is discussed. The phenomenon reported seems to differ from that observed following trauma and other forms of stress.

Schepers, G. W. ORAL THERAPY OF DIABETES MELLITUS. South African Practitioner *2*:67-68, February-March 1957.

Carbohydrate metabolism is a very complex mechanism and probably can be influenced in its different phases. Much experimental research remains to be done until a final explanation is reached. One fact, however, seems clear: that the oral antidiabetic agents, at least partially, act differently from insulin. The glucose metabolism in muscle tissue increases under insulin, but not under oral antidiabetic agents. Artosin and Nadisan are not substitutes for insulin, but rate as valid therapeutic agents in their own right. There are insulin-resistant cases which may be successfully treated with oral preparations.

Sharma, Raghunath Chandra; Singh, Gurbachan; and Singh, Man Mohan (Dept. of Pathol. and Biochem., Med. Coll., Amritsar, India): RENAL EFFICIENCY IN DIABETES MELLITUS. J. Indian M. A. *28*:339-42, April 16, 1957.

Forty diabetics and twenty normal persons were investigated for renal function. It was found that renal function was normal in diabetics below fifty years of age and with diabetes of shorter duration. Gradual renal failure was observed in cases of mild to moderate diabetes of long duration who were above fifty years of age. The impaired renal function correlated well with the duration of diabetes. Albuminuria, with or without hypertension, was present more often in patients above fifty years of age who had diabetes of long duration.

Spencer, A. G.; and Morgans, M. E. (Medical Unit, Univ. College Hosp., Medical Sch., London, England): LENTE INSULIN —FOUR YEARS' EXPERIENCE. Lancet *2*:1013-17, Nov. 17, 1956.

The experience with 200 patients on Lente insulin over a four-year period is given. The data presented suggest that in this group 83 per cent could be well-controlled on a single, daily injection of Lente. Changes in insulin dosage when transfer was made from other insulins are presented. Only one case of fat atrophy was observed.

Statti, Louis W. (St. Francis and St. Joseph Hosps. and USPHS, Pittsburgh, Pa.): DIABETES: THE PATIENT, THE GENERAL PRACTITIONER, AND THE OPHTHALMOLOGIST. Clin. Med. *4*:165-67, February 1957.

Rapid refractive changes may be the first subjective sign of diabetes. Ocular paralysis with diplopia lasts two or three months, then clears up completely. New vessel formations on the iris surface (rubeosis iridis diabetica) are a serious complication; the prognosis is poor, both for vision and retention of the globe. Lens changes in the older diabetic are treated as ordinary senile cataracts, with the same results. Optic nerve changes (atrophy) are due to vascular changes rather than to an increase in blood sugar. The degree of diabetic retinopathy is related to the duration of the disease and not to its severity. Visual damage depends on macular involvement.

Statti, Louis W. (St. Francis and St. Joseph Hosps., Pittsburgh, Pa.): OPHTHALMIC PROBLEMS IN DIABETES. Geriatrics *11*: 263-65, June 1956.

Loss of vision in diabetes can be avoided or retarded for many years when there is rigid control of diet and scientifically regulated administration of insulin. Although the management of diabetes is primarily in the hands of the internist, many diabetics are first discovered by the ophthalmologist on routine eye examinations. More ocular complications of diabetes are seen today because of the increased longevity of these patients since the advent of insulin.

Stevenson, A. E. M. (Dept. of Midwifery, Queens Univ., Belfast, Ireland): PREGNANCY COMPLICATED BY DIABETES MELLITUS: A REVIEW OF 119 CASES. Brit. M. J. *2*:1514-18, Dec. 29, 1956.

The author reports on 119 diabetic pregnancies treated in the Royal Maternity Hospital, Belfast, during the sixteen-year period immediately preceding the report. Total fetal mortality was 30 per cent; total fetal loss, excluding abortions, 25 per cent. The author feels that hormone therapy in its present form is of no value and that there is, at present, no known method which will prevent intra-uterine death and allow pregnancy to go to term. Avoidable factors contributing to high fetal mortality are discussed.

Strässle, R.; and Pletscher, A. (Chemical and Medical Res. Dept. of Hoffman-La Roche & Co., A.G., Basle, Switzerland): ON THE INHIBITION OF INSULINASE BY SULFONYLUREAS. Klin. Wchnschr. *35*:719-22, July 15, 1957.

In an attempt to clarify the mechanism of insulinase-inhibition by the hypoglycemic sulfonylureas, the investigators compared this process with the insulinase-inhibition by substances with known mechanisms of action. As such they selected iodineacetamide and L-cystin, which are known to inactivate protein-sulfhydryl complexes, the former by irreversible blocking, the latter by reversible oxidation. Such comparison seemed indi-

cated particularly since the insulinase does not seem to be a homogeneous substance, but appears to consist of a thermolabile enzymatic factor and a thermostable nonenzymatic factor which is void of protein and contains sulfhydryl groups. The experiments were expected to give information as to whether the sulfonylureas act on both or only one of these factors. In this respect, moreover, it seemed desirable also to study the effect of the sulfonylureas on the glutathione-induced degradation of insulin. Glutathione is thought to act by breaking the disulfide-bridges of the insulin molecule, a process which can probably be considered as the prototype of insulin degradation by the thermostable factors of insulinase. The experiments were carried out with I^{131}-tagged insulin. Insulinase was prepared from rat liver extracts and two fractions were precipitated. Both contained the proteolytic principle but one fraction represented the precipitate of 10—60 per cent ethanol at —5°C. and *p*H 7.30 and contained 84 per cent of the substance and 95 per cent of the total activity; the other was precipitated by 40—60 per cent ethanol at —5°C. and *p*H 7.20; it contained 14 per cent of the substance and 50 per cent activity. The results of the experiments showed that the inhibition of insulinase by sulfonylureas (carbutamide and tolbutamide) varied depending on concentration of substrate. The inhibition was competitive at high insulin concentrations (62.5-2,500 $\lambda/2$ ml.). It was noncompetitive at low insulin concentrations up to 25 $\lambda/1.5$ ml. A saturation value of inhibition of not more than 50 per cent was obtained which could not be increased by increasing the sulfonylurea concentration. Iodineacetamide and L-cystine proved to be far stronger inhibitors than the sulfonylureas.

The glutathione degradation of insulin increased with increasing concentrations of insulin and glutathione. It was not affected by the sulfonylureas. It is concluded that the sulfonylureas inhibit only the enzymatic part of the insulinase system. The mechanism of this inhibition differs probably from that of iodine-acetamide and L-cystine which also affect the nonenzymatic insulinase factor. Experiments on the effect of these latter substances on insulin or on the glutathione degradation of insulin, which might have yielded interesting contributory results, are not included.

Strauzenberg, S. E. (Medizinische Akademie, Dresden, Germany): EFFECTS OF A PERORALLY ADMINISTERED NORADRENALIN PREPARATION ON THE CARBOHYDRATE ALTERATION IN DIABETES MELLITUS. München. med. Wchnschr. *99*: 260-62, February 1957.

The observation is confirmed by the author that about half of the diabetics respond with blood-sugar increase to administration of a preparation with noradrenalin effect. Furthermore, it was noted that even after peroral administration of such a preparation (Novadral), approximately half of the examined diabetics reacted with increased glycosuria. This increase of glycosuria is—in opposition to hitherto held conceptions—not independent of metabolism.

Sugar, Samuel J. N. (George Washington University Sch. of Medicine, Washington, D. C.): CLINICAL EXPERIENCE WITH MORE THAN 100 PATIENTS TREATED WITH ORINASE. M. Ann. District of Columbia *26*:293-96, June 1957.

The exact mode of action of Orinase is not completely known. Experimental evidence indicates probable stimulation of the beta cells of the pancreas. Seventy-five per cent of the 131 patients studied were satisfactorily controlled with Orinase. No serious side effects were observed. Most prominent was a tendency to gain weight. Best results were noted in middle-aged patients with relatively recent onset of diabetes requiring less than 40 units of insulin. Orinase is not an insulin substitute. When ketosis appears insulin must be used.

Sugar, Samuel J. N.; Levitsky, Leon R.; and Bergemann, Till (Med. Dept. of Prince George's Genl. Hosp., Cheverly, Md.): MANAGEMENT OF DIABETES MELLITUS WITH ORINASE: A PRELIMINARY REPORT. M. Ann. District of Columbia *25*:529-33; 585, Oct. 1956.

Many adult diabetics who require daily insulin injections were controlled satisfactorily with Orinase alone. Best success was noted in adults whose diabetes was of recent onset and mild enough to require only small doses of insulin. These patients could be controlled on an ambulatory basis. Unstable diabetics of any age are poor candidates for control with Orinase. Orinase is not an insulin substitute. When evidence of ketosis appears, insulin must be used. Toxicity to Orinase is low. Its exact mode of action is not completely known. Experimental evidence indicates a prevention of destruction of insulin in the liver.

Thiel, A. (Dept. of Anat., Univ. of Kiel, Kiel, Germany): THE HISTO-PATHOLOGY OF THE ISLETS OF LANGERHANS IN THE PANCREAS AND DIABETES MELLITUS. Deutsche med. Wchnschr. *82*:349-50, March 8, 1957.

This article is a concise and critical review of our present knowledge of structure and function of the islets of Langerhans. Facts and hypotheses are clearly distinguished. Insulin, the hormone of the beta cells, is most likely stored as a zinc complex in the beta-cell granules. Zinc appears to regulate insulin storage and secretion. The alpha cells are probably the site of glucagon production, but there is as yet no evidence that the alpha-cell granules contain glucagon or its precursor. The concept of an altered alpha beta-cell ratio in clinical diabetes mellitus is still hypothetical, and the factual evidence is contradictory. An increase of alpha cells can be found in nondiabetic individuals and may be absent in diabetics. The alphacytotoxic action of certain hypoglycemic agents, such as synthalin and IPTD, does not seem to be specific and does not exclude the possibility that the hypoglycemia is due to an extrapancreatic action. Hypoglycemic sulfonamides (BZ-55, D-860) have, if at all, only a mild alphacytotoxic action, which probably is not related to their blood sugar-lowering effect.

Thosteson, George C. (Harper Hosp., Detroit, Michigan): ORAL HYPOGLYCEMIC AGENTS. Harper Hospital Bull. *14*:323-26, September-October 1956.

Many of the oral agents for the reduction of blood sugar, developed in the past two decades, have either failed to come up to expectations, or have been outright fakes. Some of the organic (botanic) sources of hypoglycemic agents, known for some time, have recently been studied more extensively. Of the inorganic preparations, those related to the sulfonamides appear to have a limited hypoglycemic effect. No true oral substitute for insulin as yet has appeared on the therapeutic horizon.

Voll, Artur; and Kloster, Johan (Aust-Agder Central Hosp., Med. Dept., Arendal, Norway): INSULIN RESISTANCE. Acta med. scandinav. *157*:223-32, April 18, 1957.

A case of insulin resistance in a seventy-one-year-old man, who was not overweight, is reported. He had previously received small doses of insulin for several months. The requirement rose rapidly, there was a pronounced tendency to acidosis,

and, in the hospital, his diabetes was not brought under control until a total daily dose of about 400 units was used. The patient had moderate rheumatoid arthritis. The blood sedimentation rate was raised. Multiple X rays of the chest showed a right-sided infiltration considered to be a long-standing fibroid process, possibly with bronchiectasis. Otherwise no pathological changes were found clinically, radiologically or at laboratory tests, apart from a slight increase in gamma-globulins and positive Takata-Ara and thymol tests. While in hospital there was a mild local allergic reaction at the site of insulin injections. No reduction of the insulin requirements occurred when these reactions disappeared spontaneously. On investigation, using the rat diaphragm method, an insulin-inhibiting factor was demonstrated in the patient's serum. The causes of insulin resistance are discussed, and the case is considered in relation to the different possibilities.

Wallach, Stanley; Englert, Edwin, Jr.; and Brown, Harold (Veterans Administration Hosp. & Dept. of Med., Univ. of Utah Coll. of Medicine, Salt Lake City, Utah): ADRENOCORTICAL FUNCTION IN DIABETIC ACIDOSIS. Metabolism 6:107-15, March 1957.

A report of studies in five diabetic patients with adequate adrenocortical function in whom elevated plasma free and conjugated 17-hydroxycorticosteroid and increased urinary 17-hydroxycorticosteroid levels were found during diabetic acidosis. These levels, with recovery, returned promptly to normal. Delayed return to normal occurred in two who developed hypoglycemia during insulin therapy. Partition of the urine into free and conjugated fractions showed 85 per cent of the total 17-hydroxycorticosteroid excreted in the conjugated form; one patient with primary adrenal insufficiency and diabetes mellitus during ketosis and mild acidosis did not develop an elevated plasma 17-hydroxycorticosteroid level. The authors conclude that the increased adrenocortical activity seen in diabetic acidosis is a result and not a cause of the syndrome.

Weinges, K. F. (II. Medical Dept., University Munich, Germany): CLINICAL EXPERIENCES WITH A NEW ZINC-DEPOT-INSULIN. Deutsche med. Wchnschr. 82:848-51, May 24, 1957.

A new suspension of amorphous zinc insulin in a glycol buffer at neutral *p*H (Z-N-Insulinum Organon) was used. The buffer prevents precipitation of excess zinc, and permits mixing of this preparation with other insulin solutions. Patients with stable diabetes who were well controlled by diet and insulin were tested. Elderly diabetics with daily insulin requirement of about 40 units could easily be switched to the new preparation. Juvenile diabetics showed a greater sensitivity; with comparable doses they experienced hypoglycemic reactions during daytime, showed hyperglycemia and glycosuria during the night and the following morning. Thus the new suspension does not seem to be suitable for the juvenile type of diabetes. No local or general allergic reactions were observed.

Weinman, E. O.; Strisower, E. H.; and Chaikoff, I. L. (Dept. of Physiol., Univ. of California School of Med., Berkeley, Calif.): CONVERSION OF FATTY ACIDS TO CARBOHYDRATE: APPLICATION OF ISOTOPES TO THIS PROBLEM AND ROLE OF THE KREBS CYCLE AS A SYNTHETIC PATHWAY. Physiol. Rev. 37:252-72, April 1957.

The question is often posed, whether fatty acids are converted to glucose, and if so, by what pathway. Isotopic studies with labeled compounds show that fatty acid carbons become incorporated into glucose, and that, in mammals, the major pathway by which such incorporation occurs is via Acetyl-CoA, the Krebs cycle, decarboxylation of oxalacetate with the formation of phosphopyruvate, and the modified reversal of the Embden-Meyerhof scheme of glycolysis. Since from each fatty acid two carbons enter the Krebs cycle as acetyl-CoA, and two carbons are evolved as CO_2 with each turn of the cycle, no carbons are made available for synthetic reactions from this acetate influx from fatty acids, and hence there can be no net gain from fatty acid in glucose by this operation of the cycle. A scheme is presented, and characterized mathematically, by which Krebs cycle carbons become available for synthetic reactions. This is made possible by an additional nonacetate influx of Krebs cycle intermediates into this cycle. The influx not only determines the extent of synthetic reactions possible via the Krebs cycle, but also influences the degree to which carbons derived from acetate participate in these reactions. This additional, nonacetate influx into the Krebs cycle is reflected in certain ratios which can be measured. The significance of these ratios, apart from the question of the conversion of fat to carbohydrate, lies in the fact that they afford a simple means for estimation of the maximal extent of synthetic activity via the Krebs cycle. Of these, the CO_2 ratio ($C^{14}O_2$ recovered from acetate-1-C^{14}/$C^{14}O_2$ recovered from acetate-2-C^{14} is most easily obtained. In a survey of data in the literature, it is shown that the Krebs cycle functions as a synthetic as well as an oxidative pathway. The originally posed question, whether fats are converted to carbohydrates, must be considered in terms of an interrelation among the various body constituents. Whether incorporation, exchange, synthesis, or some other term properly describes this relation between fatty acids and glucose is a matter of terminology. It is quite clear, however, that fats cannot give rise to a net gain of carbohydrate in the same sense that carbohydrate yields a net gain of fat. But experiments with isotopes have served to illustrate a dynamic interrelation in which (1) acetate—and therefore fatty acids—contributes carbon to glucose formed via the Krebs cycle; (2) a nonacetate influx into the Krebs cycle makes possible the net formation of glucose via this cycle; (3) the interdependence of the above processes is reflected quantitatively in certain ratios, such as the one showing the recovery of labeled CO_2 from carboxyl relative to that from methyl labeled acetate. These indicate a net gain of glucose via the Krebs cycle possibly from glucogenic amino acids and from known gluconeogenic Krebs cycle intermediates such as succinic acid.

Weiss, T. E.; Segaloff, A.; and Moore, Charles (Dept. of Int. Med., Ochsner Clin. & Tulane University Sch. of Med., New Orleans, La.): GOUT AND DIABETES. Metabolism 6:103-06, March 1957.

During evaluation of a large group of cases of gout, frequent elevations in blood sugar were noted. Others have observed this in the past, including a strong diabetic family history in gouty individuals. Of 143 patients with gout, 28 per cent showed elevations in blood sugar levels. In a control group, matched for age, sex and obesity, 15 per cent had abnormal blood sugar levels. The authors conclude that at least a 50 per cent greater incidence of abnormal carbohydrate metabolism is present in gouty than in nongouty individuals. However, only one had diabetes of sufficient degree to require insulin. Certain features common to both diseases have been reviewed. They postulate that hyperuricemia in a patient with familial tendency to diabetes has an ameliorating effect on its clinical expression.

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Sixth Postgraduate Course in Diabetes and Basic Metabolic Problems

Atlanta, Georgia, Jan. 22-24, 1958

The American Diabetes Association will hold its Sixth Postgraduate Course in Diabetes and Basic Metabolic Problems at the Academy of Medicine of the Fulton County Medical Society in Atlanta, Georgia, Jan. 22-24, 1958. Lectures will be given in the Academy's auditorium.

Christopher J. McLoughlin, M.D., of Emory University School of Medicine and Consultant in Diabetes at Grady and Piedmont Hospitals in Atlanta, is Director of the Course. The Associate Directors are C. Raymond Arp, M.D., of Emory University School of Medicine, and Walter L. Bloom, M.D., Director of Medical Education and Research at Atlanta's Piedmont Hospital.

The Course was developed by the Committee on Professional Education of the American Diabetes Association and is open to members of the medical profession.

A Social Hour and Banquet are scheduled for Wednesday evening, January 22, at the Atlanta Biltmore Hotel. Registrants and faculty members are guests of the American Diabetes Association at the Banquet and the Social Hour is by individual subscription. Another Social Hour will be held on Thursday evening, January 23, at 6:00 p.m. Later that evening, there will be a public meeting of the Diabetes Association of Atlanta.

FEES: The registration fee for the three-day Course is $40 for members of the American Diabetes Association and $75 for nonmembers. The full fee must be paid when the Course application is filed.

Fellows and residents engaged in full-time study of

medicine and allied sciences in schools and hospitals in Atlanta's metropolitan area may attend the scientific sessions at the nominal fee of $10; interns may attend without charge.

REGISTRATION: An application form is attached to the preliminary program that has been mailed to members and subscribers to the Journal. It should be filled out and mailed, with the fee, to the National Office of the American Diabetes Association as soon as possible. Applications will be accepted in the order received and registrations will be officially confirmed.

HOTEL ACCOMMODATIONS: The Atlanta Biltmore will serve as the headquarters hotel. Reservation cards will be sent to registrants with their matriculation card confirming acceptance for the Course.

POSTGRADUATE CREDIT: The American Academy of General Practice will give twenty hours' Postgraduate Credit for the Course.

THE FOLLOWING TOPICS AND DISCUSSIONS (SUBJECT TO CHANGE) WILL BE COVERED DURING THE 3-DAY COURSE.

WEDNESDAY MORNING, JANUARY 22

8:00 Registration
9:15 Introduction and Welcome—Christopher J. McLoughlin, Director

HORMONAL INFLUENCES IN DIABETES MELLITUS: Chairman, John A. Reed

9:25 Pituitary: *Basic Mechanisms*—Alfred E. Wilhelmi
9:45 *Clinical Applications*—E. Perry McCullagh
10:05 Adrenals: *Basic Mechanisms*—Jane A. Russell
10:25 *Clinical Applications*—Robert H. Williams
10:45 *Intermission*
10:55 Pancreas: *Basic Mechanism*—Arnold Lazarow
11:15 *Insulin*—Charles H. Best
11:35 *Glucagon*—Charles H. Best
11:55 Nonhormonal Factors Influencing Insulin Action—Robert H. Williams
12:15 Question and Answer Period

WEDNESDAY AFTERNOON, JANUARY 22

SPECIAL CONSIDERATIONS IN DIAGNOSIS: Chairman, Alexander Marble

2:00 Clinical History—Joseph T. Beardwood, Jr.
2:20 Laboratory Methods—Harvey C. Knowles, Jr.
2:40 Retinopathy—Albert A. Brust
3:00 *Intermission*
3:10 Neuropathy—Henry T. Ricketts
3:40 Nephropathy—Arthur J. Merrill
4:10 Errors in Diagnosis—John H. Warvel, Sr.
4:40 Question and Answer Period

WEDNESDAY EVENING, JANUARY 22

6:30 Social Hour
7:30 Banquet. Mr. Leo Aikman, Columnist, *Atlanta Constitution,* will speak.

THURSDAY MORNING, JANUARY 23

TREATMENT: Chairman, William H. Olmsted

9:00 Diets: Selecting the Diet and Instructing the Patient—Alexander Marble
9:20 Use of Insulins in the Treatment of Diabetes—Franklin B. Peck, Sr.
9:50 Oral Hypoglycemic Compounds: Physiological Mechanisms of Action—Rachmiel Levine
10:20 *Intermission*
10:30 Oral Hypoglycemic Compounds: Clinical Usefulness—Garfield G. Duncan
11:00 Insulin Resistance—Francis D. W. Lukens
11:30 A Diabetic Clinic—Leon S. Smelo
12:00 Instruction of Students, Interns and Residents—Perry S. MacNeal

THURSDAY AFTERNOON, JANUARY 23

PROBLEMS ENCOUNTERED IN THE TREATMENT OF DIABETES: Chairman, Francis D. W. Lukens

2:00 The Adolescent Diabetic—Harvey C. Knowles, Jr.
Discusssion—Robert L. Jackson
2:40 Atherosclerosis: A Survey—Henry T. Ricketts
3:00 *Intermission*
3:10 Vascular Complications in the Diabetic—W. Wallace Dyer

3:30 Hypertensive and Cardiac Complications—Arthur M. Knight, Jr.
3:50 The Overtreated Diabetic—Alexander Marble
4:15 *Panel Discussion*—Henry T. Ricketts, Moderator; Harvey C. Knowles, Jr., Robert L. Jackson, W. Wallace Dyer, Arthur M. Knight, Jr., Alexander Marble

THURSDAY EVENING, JANUARY 23

6:00 Social Hour
8:00 Public Meeting—Diabetes Association of Atlanta

FRIDAY MORNING, JANUARY 24

STRESS AND STRAIN: Chairman, Henry T. Ricketts

9:00 Management of the Surgical Diabetic—Frederick W. Williams
9:20 Infections—William F. Friedewald
9:40 Emotional Aspects—Paul L. Schroeder
10:00 The Obese Diabetic—Francis D. W. Lukens
10:20 *Intermission*

Symposium: *Socio-economic Problems of the Diabetic*

10:30 Fitting the Diet to the Income—Louis K. Alpert
11:00 Marriage and Parenthood—Edwin W. Gates
11:30 Rehabilitation and Employment—Joseph T. Beardwood, Jr.
12:00 Life Insurance for Diabetics—Mr. J. E. K. Kennedy

FRIDAY AFTERNOON, JANUARY 24

STRESS AND STRAIN: Chairman, Franklin B. Peck, Sr.

2:00 Alterations in Protein, Carbohydrate and Fat Metabolism—T. S. Danowski
2:20 Acidosis: Alterations in Electrolytes and Hemodynamics—Thomas Findley
2:40 Rapid Quantitative Determination of Blood Ketones—Walter L. Bloom
3:00 *Intermission*
3:10 The Pregnant Diabetic—E. Paul Sheridan
3:30 Summarizing Stress and Strain—Arnold Lazarow
3:50 *Panel Discussion*—Frederick W. Williams, Moderator; William F. Friedewald, Louis K. Alpert, Edwin W. Gates, Joseph T. Beardwood, Jr., Thomas Findley, Walter L. Bloom, and Arnold Lazarow.
4:30 Prophecies About Diabetes—Charles H. Best

FACULTY OF THE SIXTH POSTGRADUATE COURSE

LOUIS K. ALPERT, M.D., *Clinical Professor of Medicine, George Washington University School of Medicine, Washington, D.C.*

JOSEPH T. BEARDWOOD, JR., M.D., *Professor of Metabolic Diseases, Graduate School of Medicine of the University of Pennsylvania; and Director, Metabolic Division, Graduate Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania.*

CHARLES H. BEST, C.B.E., M.D., F.R.S., *Professor of Physiology; Director and Professor in the Banting and Best Department of Medical Research, University of Toronto, Toronto, Ontario, Canada.*

WALTER L. BLOOM, M.D., *Director of Medical Education and Research, The Piedmont Hospital, Atlanta, Georgia.*

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Heart Clinic; Chief of Medicine, Memorial Hospital, Waycross, Georgia.

HARVEY C. KNOWLES, JR., M.D., *Associate Professor of Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio.*

ARNOLD LAZAROW, M.D., *Professor and Head, Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.*

RACHMIEL LEVINE, M.D., *Professorial Lecturer in Physiology, University of Chicago; Chairman, Department of Medicine and Director, Metabolic and Endocrine Research, Michael Reese Hospital, Chicago, Illinois.*

F. D. W. LUKENS, M.D., *Professor of Medicine and Director, George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pennsylvania.*

E. PERRY MCCULLAGH, M.D., *Head, Department of Endocrinology and Metabolism, Cleveland Clinic, Cleveland, Ohio.*

PERRY S. MACNEAL, M.D., *Associate Professor of Clinical Medicine, University of Pennsylvania Medical School; Physician, Pennsylvania Hospital and the Benjamin Franklin Clinic, Philadelphia, Pennsylvania.*

ALEXANDER MARBLE, M.D., *Assistant Clinical Professor of Medicine, Harvard Medical School; Joslin Clinic and New England Deaconess Hospital, Boston.*

ARTHUR J. MERRILL, M.D., *Associate Professor of Clinical Medicine, Emory University School of Medicine; Visiting Physician, Piedmont, Emory University and Grady Memorial Hospitals, Atlanta, Georgia.*

WILLIAM H. OLMSTED, M.D., *Associate Professor of Clinical Medicine (Emeritus), Washington University School of Medicine; Barnes Hospital, St. Louis, Missouri.*

FRANKLIN B. PECK, SR., M.D., *Associate Professor of Medicine, Indiana University School of Medicine; Consultant in Medicine, Indianapolis General Hospital, Indianapolis, Indiana.*

JOHN A. REED, M.D., *Assistant Clinical Professor of Medicine, George Washington University School of Medicine; Attending Physician, George Washington University Hospital, Washington, D.C.*

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LEON S. SMELO, M.D., *Assistant Professor of Clinical Medicine, Medical College of Alabama; Consultant in Metabolic Diseases, Children's Hospital of Birmingham; Medical Staff, Birmingham Baptist Hospitals, University Hospital and St. Vincent's Hospital, Birmingham, Alabama.*

JOHN H. WARVEL, SR., M.D., *Assistant in Medicine, Indiana University School of Medicine and Diabetic Clinic, Methodist Hospital, Indianapolis, Indiana.*

ALFRED E. WILHELMI, PH.D., *Professor of Biochemistry, Emory University School of Medicine, Emory University, Georgia.*

FREDERICK W. WILLIAMS, M.D., *Associate Clinical Professor of Medicine, New York Medical College; Morrisania City Hospital, New York, New York.*

ROBERT H. WILLIAMS, M.D., *Professor and Executive Officer, University of Washington School of Medicine, Seattle, Washington.*

EIGHTEENTH ANNUAL MEETING

As previously announced, the Eighteenth Annual Meeting of the American Diabetes Association will be held at the Hotel Mark Hopkins in San Francisco, June 21-22, 1958, immediately prior to the Annual Session of the American Medical Association, June 23-27. Members of the Association are urged to review the communication sent to them on Nov. 21, 1957, by John A. Reed, M.D., President, particularly with reference to hotel reservations. If the letter is not readily available another copy may be secured by writing to the national office.

Jerome W. Conn, M.D., Professor of Medicine, Division of Endocrinology and Metabolism and the Metabolic Research Unit, University of Michigan, will deliver the Banting Memorial Lecture.

Physicians and other scientists, who would like to present papers at the Scientific Sessions, are invited to submit abstracts to Francis D. W. Lukens, M.D., Chairman of the Committee on Scientific Programs. Eleven copies of the abstracts, which are requested in order to expedite review by the Committee, should be sent to Dr. Lukens in care of the national office. *Abstracts still may be considered if received before March 1, 1958.*

BOARD OF STATE GOVERNORS

Lewis B. Flinn, M.D., of Wilmington, Delaware, was elected Chairman of the Board of State Governors on June 1, 1957, at the time of the Seventeenth Annual Meeting. He succeeds Edgar A. Haunz, M.D., of Grand Forks, North Dakota, and by reason of this office will serve as an ex officio member of the Council of the American Diabetes Association.

Other newly-elected officers include Morris Margolin, M.D., of Omaha, Nebraska, who was elected Vice Chairman, and Albert S. Easley, M.D., of Chattanooga, Tennessee, who was elected Secretary of the Board.

The following Governors will serve for the organizational year 1957-58.

Term Expiring June 1960

State	*Governor*
Alabama	Samuel Eichold, M.D.
Arizona	Eleanor Waskow, M.D.
Arkansas	Hal Dildy, M.D.
California	
Northern & Nevada	Leo Goodman, M.D.
Southern	Roy F. Perkins, M.D.
Colorado	W. Bernard Yegge, M.D.
Connecticut	Barnett Greenhouse, M.D.
Delaware	Lewis B. Flinn, M.D.
District of Columbia	Maurice Protas, M.D.
Florida	Sidney Davidson, M.D.
Georgia	Christopher J. McLoughlin, M.D.
Idaho	Glenn Q. Voyles, M.D.
Illinois	
Northern	Ford K. Hick, M.D.
Southern	Thomas D. Masters, M.D.
Indiana	Marion R. Shafer, M.D.
Iowa	Robert C. Hardin, M.D.

Term Expiring June 1958

Kansas	Thomas J. Luellen, M.D.
Kentucky	Carlisle Morse, M.D.
Louisiana	Daniel W. Hayes, M.D.
Maine	Elton R. Blaisdell, M.D.
Maryland	Ernest C. Brown, Jr., M.D.
Massachusetts	Reed Harwood, M.D.
Michigan	Laurence F. Segar, M.D.
Minnesota	Moses Barron, M.D.
Missouri	Henry E. Oppenheimer, M.D.
Montana	Felix H. Crago, M.D.
Nebraska	Morris Margolin, M.D.
Nevada	See Northern California
New Hampshire	See Vermont
New Jersey	Benjamin Saslow, M.D.
New York, Western	George F. Koepf, M.D.
North Carolina	Charles W. Styron, M.D.
North Dakota	Edgar A. Haunz, M.D.

Term Expiring June 1959

Ohio	Joseph I. Goodman, M.D.
Oklahoma	Bert F. Keltz, M.D.
Oregon	Wm. Richey Miller, M.D.
Pennsylvania	
Eastern	William F. Hanisek, M.D.
Western	John A. O'Donnell, M.D.
Rhode Island	Louis I. Kramer, M.D.
South Carolina	Robert Wilson, M.D.
South Dakota	Everett W. Sanderson, M.D.
Tennessee	Albert S. Easley, M.D.
Texas	George M. Jones, M.D.
Utah	Donald E. Smith, M.D.
Vermont &	
New Hampshire	Ellsworth L. Amidon, M.D.
Virginia	William R. Jordan, M.D.
Washington	Robert L. Reeves, M.D.
West Virginia	Richard N. O'Dell, M.D.
Wisconsin	Elwood W. Mason, M.D.

ADLER FOUNDATION MAKES GRANT FOR THREE-YEAR RESEARCH FELLOWSHIP

The American Diabetes Association is pleased to announce that the Adler Foundation has made a grant for a three-year Fellowship to be known as the "Adler Foundation Research Fellowship."

The Committee on Research and Fellowships requested funds several years ago for establishment of such long-term training, and this grant from the Adler Foundation of approximately $15,000 for three years now makes it possible to begin this important phase of our Research Fellowships Program.

MEAL PLANNING PUBLICATIONS

"Meal Planning with Exchange Lists," a booklet prepared to help diabetics select foods for their meals, is available at $.15 each; $6.50 per 100 copies and $50.00 per 1,000 copies. Meal Plans No. 1 through No. 9, "ADA Bland Low-fiber Diabetic Diet" and "ADA Sodium Restricted Diabetic Diet," may be secured at $.05 per single copy; $2.00 per 100 copies; $18.00 per 1,000 copies. Order forms are available from the American Diabetes Association, 1 East 45th St., New York 17, N. Y.

1957-58 MEDICAL STUDENT-INTERN ESSAY CONTEST

Medical students, interns and physicians within two years after their graduation from medical school are invited to enter the sixth Medical Student-Intern Essay

Contest sponsored by the American Diabetes Association. Any subject relating to diabetes and basic metabolic problems may be selected.

The author or authors of the best paper reporting original work, whether laboratory investigation or clinical observation, will receive an award of $250. This prize is again made possible through the kindness of the St. Louis Diabetes Association.

The author or authors of the best review article or case report will be awarded a prize of $50.

Papers will be judged on the basis of value of the material and method of presentation.

Members of the American Diabetes Association and subscribers to DIABETES are requested to encourage medical students and interns to enter the contest. Entrants should submit the original and two copies of their manuscript. The manuscript should be typewritten and double-spaced, and mailed by April 1, 1958, to: Committee on Scientific Awards, American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

IDF HOLDS THIRD CONGRESS

As previously announced, members of the American Diabetes Association and its Affiliates may be able to fly by chartered plane at reduced rates to attend the next Congress of the International Diabetes Federation at Düsseldorf, Germany. Requests for passage on the chartered plane must be received not later than Feb. 1. The Congress will be held July 21-25, 1958.

Howard F. Root, M.D., Vice President of IDF, requests those interested to address inquiries and reservations to the Manning Travel Bureau, Hotel Kenmore, Commonwealth Avenue, Boston 15, Massachusetts. Additional information and application forms were sent November 21 to members of the American Diabetes Association.

DIABETES ABSTRACTS

A limited number of back issues of DIABETES ABSTRACTS are available upon request to the American Diabetes Association, 1 East 45th St., New York 17, N.Y. These issues include: Volume 5, Number 4 (October 1946), which contains the five-year cumulative index for Volumes 1-5; Volume 10, Number 1 (January 1951); and Volume 4, Number 3 (July 1945).

ADA IDENTIFICATION CARD

An identification card for diabetics, issued by the American Diabetes Association, is available. As announced previously, this card was developed by the Committee on Information for Diabetics, bears the official seal of the Association, and will fit the average pocket or purse wallet. The price is $.10 each in quantities of one through nine and $.05 each in quantities of ten or more, both prices including handling and shipping.

BINDERS FOR VOLUME 7 NOW AVAILABLE

A binder for 1958 matching those for Volumes 1, 2, 3, 4, 5 and 6 (1952-57 issues) of DIABETES is available for immediate shipment at a price of $2.25. This insert binder is sturdy and attractive and will hold six issues of the magazine. Readers are urged to purchase theirs before present stocks are depleted. Binders for Volumes 1, 2, 3, 4, 5 and 6 also are available at the same price. Address your orders to American Diabetes Association, Inc., 1 East 45th St., New York 17, N.Y.

NEW MEMBERS

Active

The following were elected as of Dec. 1, 1957, and Jan. 1, 1958:

Alabama
Weil, Warren B. — Birmingham

California
Walworth, David H. — San Jose

Florida
Hills, Arthur G. — Miami

Georgia
Kravtin, Abraham J. — Columbus
Owen, John A., Jr. — Augusta
Smith, G. Bertling — Columbus

Illinois
Wool, Ira G. — Chicago

Indiana
Langrall, Harrison M. — Marion

Iowa
Stone, Daniel B. — Iowa City

Maryland
Prout, Thaddeus E. — Baltimore

Massachusetts
Boshell, Buris R. — Boston

Minnesota
McClure, Rensselaer W., Jr. — Rochester
Wakai, Coolidge S. — Rochester

New York
Brothers, Milton J. — New York
Gershberg, Herbert — New York
Neuberg, Hans W. — New York

Other Countries

Belgium
DeMeutter, Roger C. — Brussels

Canada
Kowalewski, K. P. — Edmonton, Alberta

Japan
Bush, Ovid B., Jr. — Osaka

Associate

New York
Ostrowsky, Marilyn — New York

NEWS OF AFFILIATE ASSOCIATIONS

The FLORIDA DIABETES ASSOCIATION held its Fifth Annual Session Oct. 24-26, 1957, at the College of Medicine, University of Florida, Gainesville, Florida. Guest speakers included William R. Jordan, M.D., Richmond, Virginia; C. J. O'Donovan, M.D., Kalamazoo, Michigan, and Roger Unger, M.D., Dallas, Texas. Member speakers included Sidney Davidson, M.D., Lake Worth, Florida, Sanford A. Mullen, M.D., Jacksonville, Florida, and William C. Thomas, Jr., M.D., Gainesville, Florida.

The NEW ENGLAND DIABETES ASSOCIATION will hold its Winter Clinical Meeting in the Jimmy Fund Building Auditorium in Boston on March 5, 1958. Francis D. W. Lukens, M.D., will speak on "Insulin and Protein Metabolism." The Spring Clinical Meeting, featuring a "Symposium on Hemochromatosis," will be held at the Mary Hitchcock Hospital, Hanover, New Hampshire, on March 21. Programs of the New England Diabetes Association in 1957 included the Diabetes Fair (November 18, 19) and the Fall Clinical Meeting (December 3). The Fall Clinical Meeting included a "Symposium on Fat Metabolism and Diabetes," with the following participants: James Ashmore, Ph.D., Harvard Medical School, "Inter-relationship between Fat and Carbohydrate Metabolism"; Vincent P. Dole, M.D., Rockefeller Institute, "Ketogenesis"; Albert E. Renold, M.D., New England Deaconess Hospital, "Action of Insulin on Adipose Tissue"; Robert Schwartz, M.D., Children's Hospital, "Lipoatrophic Diabetes."

The NEW YORK DIABETES ASSOCIATION (Clinical Society) and the Section on Obstetrics and Gynecology of the New York Academy of Medicine will hold a combined meeting on "Management of the Pregnant Diabetic," Jan. 28, 1958, at 8:30 p.m. The meeting will be held at the New York Academy of Medicine Building, 2 East 103rd St., New York 29, N.Y. Irving Graef, M.D., will be moderator of the panel discussion, which will include the following participants: William P. Given, M.D., Edmund L. Shlevin, M.D., Alvin J. B. Tillman, M.D., Priscilla White, M.D., Boston, Henry Dolger, M.D., and Walter S. Jones, M.D., Providence. A general discussion will follow.

NEWS NOTES

FIFTH CONGRESS OF INTERNATIONAL SOCIETY OF INTERNAL MEDICINE

The International Society of Internal Medicine has announced that its Fifth Congress will be held at the new Sheraton Hotel, Philadelphia, April 23-26, 1958. This will be the first meeting of the Society outside of Europe. Frank N. Allan, M.D., Boston, is a member of the Executive Committee and Chairman of the Program Committee. Charles H. Best, M.D., Toronto, will participate in the program.

The scientific program will include morning lectures on newer developments in the prevention, diagnosis and treatment of disease, with an appraisal of clinical experience in various geographic areas. Among the subjects will be cardiovascular lesions that now are amenable to surgical therapy, the use of anticoagulants, physical medicine and rehabilitation, arthritis, oral remedies in the therapy of diabetes, and the diagnostic and therapeutic application of radioactive agents. During the afternoon sessions there will be panels, symposia and brief reports on various controversial subjects. Cardiovascular, hematological, viral and malignant diseases, as well as gastro-intestinal, respiratory, renal and neurological subjects will be included.

The 1958 Annual Session of the American College of Physicians will occur in Atlantic City, April 28-May 2, immediately following the International Congress.

FEDERAL FUNDS ALLOTTED

In November, the National Institutes of Health approved and awarded 1,589 research grants with an aggregate value of $27,013,630. Grantees in this group are attached to 310 non-Federal institutions in forty-five states, two territories, the District of Columbia and fourteen foreign lands. One hundred ninety-one grants totaling $2,311,178 were awarded by the National Institute of Arthritis and Metabolic Diseases.

Research grants of $46,031,816 had been approved and allocated previously by the Public Health Service during the four-month period ending Oct. 31, 1957. Total of 3,325 grants were awarded to support projects in 478 medical schools, universities, hospitals and research centers in forty-eight states, the District of Columbia, Hawaii, Alaska and eighteen foreign countries. Of the total, a sum of $7,137,003 was allotted to the study of arthritis and metabolic diseases.

STUDENT SUBSCRIPTION RATES

Medical Students, Interns and Residents are now offered a one-year subscription to DIABETES at a special price of $4.50, half of the regular subscription rate. This reduction was recommended by the Committee on Scientific Publications, and accepted by the Council of the American Diabetes Association.

PERSONALS

DWIGHT J. INGLE, PH.D., Chicago, and S. O. WAIFE, M.D., Indianapolis, are Editors of *Perspectives in Biology and Medicine*, a new quarterly journal dedicated to a multidisciplined approach to the problems of biology and medicine. Other members of the American Diabetes Association who will serve on the Advisory Board and Editorial Board respectively are B. A. Houssay, M.D., Buenos Aires, and Frank L. Engel, M.D., Durham, North Carolina.

RACHMIEL LEVINE, M.D., Chicago, HERBERT POLLACK, M.D., New York City, and THEODORE B. VAN ITALLIE, M.D., Boston, presented lectures at the 40th Annual Meeting of the American Dietetic Association, held at Miami, Florida, Oct. 22-25, 1957. Dr. Levine spoke on "Oral Hypoglycemic Agents in Treatment of Diabetes," Dr. Pollack on "Today's Concepts in Dietary Care of the Patient with Diabetes," and Dr. Van Itallie on "Latest Developments in Atherosclerosis."

Advisory committees are being appointed to supervise intramural research by National Institutes of Health. There will be eight groups, one for each of the seven National Institutes and one for the Division of Biologics Standards. ROBERT F. LOEB, M.D., New York City, and WILLIAM C. STADIE, M.D., Philadelphia, will serve on the committees for the National Heart Institute and the National Institute of Arthritis and Metabolic Diseases, respectively.

The following members of the American Diabetes Association participated in postgraduate courses arranged by the American College of Physicians during the fall:

CAMPBELL MOSES, M.D., and FRANK J. GREGG, M.D., were Co-directors of Course No. 1, "Mechanisms of Disease," held October 7-12 at the University of Pittsburgh School of Medicine. T. S. DANOWSKI, M.D., ALEXANDER D. LOWY, M.D., FRANK M. MATEER, M.D., and I. ARTHUR MIRSKY, M.D., were faculty members.

GORDON R. HENNIGAR, M.D., was a faculty member for Course No. 2, "Basic Concepts in Internal Medicine," which was held October 14-18 at the Medical College of Virginia in Richmond.

EDGAR S. GORDON, M.D., was an officer of instruction for Course No. 3, "The Biological Approaches to Internal Medicine," held October 21-25 at the University of Wisconsin Medical School in Madison.

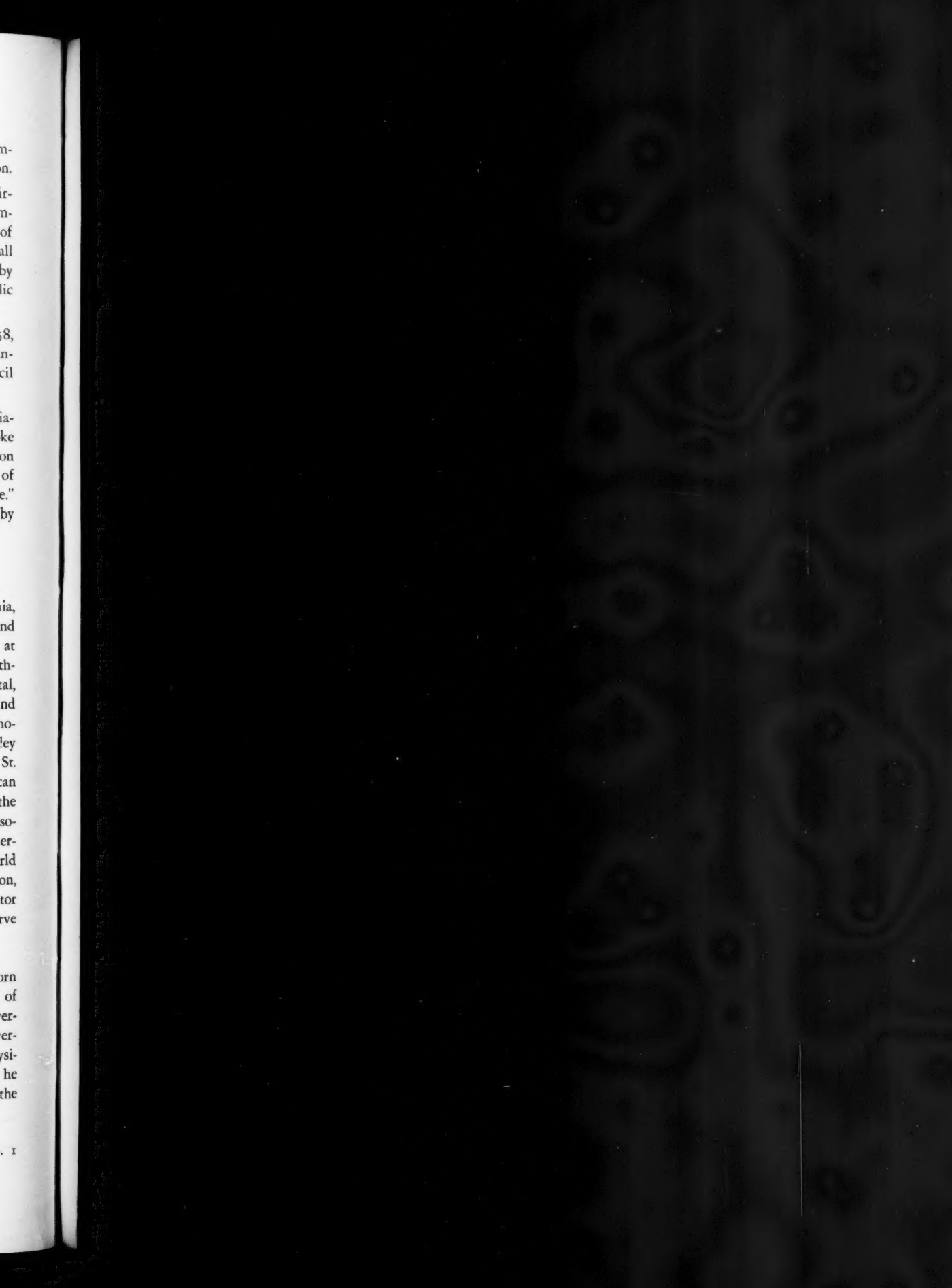
WILLIAM MCFARLAND, M.D., was an officer of instruction for Course No. 4, "Review of Recent Advances in Internal Medicine," held October 28-November 1, at Tufts University School of Medicine in Boston.

HENRY B. MULHOLLAND, M.D., Charlottesville, Virginia, was appointed to a newly formed advisory committee to assist in programming and other aspects of projects undertaken in connection with the over-all National Health Survey. The committee is chaired by Leroy E. Burney, M.D., Surgeon General of the Public Health Service.

ELMER L. SEVERINGHAUS, M.D., retired Jan. 1, 1958, as Vice President for Clinical Research of Hoffmann-La Roche, Inc. He served as a member of the Council of the American Diabetes Association 1944-48.

PRISCILLA WHITE, M.D., Boston, opened the Diabetes Detection Drive in Connecticut when she spoke at the meeting of the New Haven Medical Association on Nov. 6, 1957. Her subject was "Management of Juvenile Diabetes with Insulin and Oral Substance." The meeting was preceded by a dinner attended by Connecticut physicians.

OBITUARIES

AARON S. CANTOR, M.D., Scranton, Pennsylvania, was born Dec. 24, 1885, in Szagaren, Lithuania, and died at the age of sixty-eight. He was Cardiologist at Mercy Hospital, Scranton, and a founder of Northeastern Hospital, Philadelphia. At Hahnemann Hospital, Scranton, he was Chief of Cardiovascular Diseases, and served as Consulting Physician, Wayne County Memorial Hospital, Honesdale; Visiting Physician, Mid-Valley Hospital, Peckville; and Consulting Cardiologist, St. Mary's Hospital, Scranton. A member of the American Diabetes Association since 1941, he belonged to the American College of Chest Physicians, American Association of Anatomists, American Trudeau Society, American Association for the Advancement of Science, World Medical Association, American Geriatrics Association, and the American College of Cardiology. Dr. Cantor served as a Captain in the U.S. Medical Corps Reserve from 1918 to 1924.

CHARLES A. LAMONT, M.D., Canton, Ohio, was born in Albion, New York, in 1880 and died at the age of seventy-seven. He received his B.A. from Yale University in 1905, and his M.D. from Johns Hopkins University in 1908. In 1926, Dr. LaMont attended the Physiatric Institute at Morristown, New Jersey, where he specialized in diabetes. He had been a member of the American Diabetes Association since 1941.